

EXHIBIT G

77

What is claimed is:

1. A recombinant immunotoxin comprising an antibody or antigen-binding fragment thereof comprising SEQ ID NO:3 and SEQ ID NO:10 and a toxin moiety.

2. A recombinant immunotoxin comprising an antibody or antigen-binding fragment thereof comprising SEQ ID NO:5 and SEQ ID NO:12 and a toxin moiety.

3. A recombinant immunotoxin comprising an antibody or antigen-binding fragment thereof comprising SEQ ID NO:7 and SEQ ID NO:14 and a toxin moiety.

4. A recombinant immunotoxin of any one of claims 1, 2 or 3, wherein the toxin moiety is selected from the group consisting of *Pseudomonas* exotoxin A ("PE") or a cytotoxic fragment or mutant thereof, Diphtheria toxin or a cytotoxic fragment or mutant thereof, ricin or a cytotoxic fragment

78

thereof, abrin or a cytotoxic fragment thereof, saporin or a cytotoxic fragment thereof, pokeweed antiviral toxin or a cytotoxic fragment thereof, and bryodin 1 or a cytotoxic fragment thereof.

5. A recombinant immunotoxin of claim 4, wherein the toxin moiety is selected from the group consisting of PE38, PE35, PE40, PE4E, and PE38QQR.

6. A composition comprising a recombinant immunotoxin of claim 1 in a pharmaceutically acceptable carrier.

7. A composition comprising a recombinant immunotoxin of claim 2 in a pharmaceutically acceptable carrier.

8. A composition comprising a recombinant immunotoxin of claim 3 in a pharmaceutically acceptable carrier.

* * * * *

-continued

<400> SEQUENCE: 6

Gly Val Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 1 5 10 15

<210> SEQ ID NO 7

<211> LENGTH: 4

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:peptide linker

<400> SEQUENCE: 7

Gly Gly Gly Ser
 1

<210> SEQ ID NO 8

<211> LENGTH: 4

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:carboxyl terminus

<400> SEQUENCE: 8

Lys Asp Glu Leu
 1

<210> SEQ ID NO 9

<211> LENGTH: 4

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:carboxyl terminus

<400> SEQUENCE: 9

Arg Glu Asp Leu
 1

What is claimed is:

1. A method for inhibiting the growth of a malignant cell 45
 expressing mesothelin on its cell surface, said method comprising:

contacting said malignant cell with an effective amount of
 an immunoconjugate comprising a therapeutic agent
 attached to an anti-mesothelin antibody having six 50
 complementarity determining regions (CDRs), which
 CDRs have the sequences shown for the respective
 CDRs set forth in FIG. 1 and which specifically binds
 to cells expressing mesothelin on their cell surface

wherein said therapeutic agent inhibits the growth of said
 cell.

2. A method of claim 1, wherein said anti-mesothelin
 antibody is a single chain Fv (scFv) antibody comprising a
 variable heavy (V_H) region and a variable light (V_L) region. 60

3. A method of claim 2, wherein said V_H region is peptide
 bonded to said V_L region through a linker peptide.

4. A method of claim 2, wherein the sequence of the
 variable heavy (V_H) region is as set forth in FIG. 1.

5. A method of claim 2, wherein the sequence of the
 variable light (V_L) region is as set forth in FIG. 1.

6. A method of claim 2, wherein said scFv comprises a
 variable heavy (V_H) region and a variable light (V_L) region
 having the sequences shown in FIG. 1.

7. A method of claim 1, wherein said therapeutic agent is
 a toxin.

8. A method of claim 7, wherein said toxin is a *Pseudomo-*
nas exotoxin (PE) or a cytotoxic fragment thereof.

9. A method of claim 8, wherein said PE is PE38.

10. A method of claim 1, wherein said malignant cell is
 contacted in vivo.

11. A method of claim 1, wherein said malignant cell is
 selected from the group consisting of a mesothelioma cell,
 an ovarian cancer cell, a stomach cancer cell and a squamous
 cell cancer cell.

* * * * *

-continued

```

<210> SEQ ID NO 35
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:amplifying
      oligomer

<400> SEQUENCE: 35

gtgagtgaga attcatgcat ctctcggcc cctg                               34

<210> SEQ ID NO 36
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:amplifying
      oligomer

<400> SEQUENCE: 36

tcactcactc gcggcgctt cgctgccga tggctctc                             37

<210> SEQ ID NO 37
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:wild type
      RFB4 variable heavy chain (V-H) CDR3

<400> SEQUENCE: 37

catagtggt acggtagtag ctacggggtt ttgtttgctt ac                       42

<210> SEQ ID NO 38
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:wild type
      RFB4 variable heavy chain (V-H) CDR3

<400> SEQUENCE: 38

His Ser Gly Tyr Gly Ser Ser Tyr Gly Val Leu Phe Ala Tyr
 1             5             10

```

What is claimed is:

1. An isolated anti-CD22 antibody with a variable light (V_L) chain having the sequence of the V_L chain of antibody RFB4 and a variable heavy (V_H) chain having the sequence of the V_H chain of antibody RFB4, provided that residues 100, 100A and 100B of CDR3 of the V_H chain of said anti-CD22 antibody, as the residues of the V_H chain are numbered in the column of FIG. 3 according to "Kabat Numbering System", have an amino acid sequence selected from the group consisting of: THW, YNW, TTW, and STY.
2. An antibody of claim 1, wherein said antibody is selected from the group consisting of an scFv, a dsFv, a Fab, or a $F(ab')_2$.
3. A composition comprising an antibody of claim 1 conjugated or fused to a therapeutic moiety or a detectable label.
4. A composition of claim 3, wherein the therapeutic moiety is selected from the group consisting of a cytotoxin, a drug, a radioisotope, or a liposome loaded with a drug or a cytotoxin.

5. A composition of claim 4, wherein the therapeutic moiety is a cytotoxin.

6. A composition of claim 5, wherein the cytotoxin is selected from the group consisting of ricin A, abrin, ribotoxin, ribonuclease, saporin, calicheamycin, diphtheria toxin or a cytotoxic subunit or mutant thereof, a *Pseudomonas* exotoxin, a cytotoxic portion thereof, a mutated *Pseudomonas* exotoxin, a cytotoxic portion thereof, and botulinum toxins A through F.

7. A composition of claim 6, wherein said cytotoxin is a *Pseudomonas* exotoxin or cytotoxic fragment thereof, or a mutated *Pseudomonas* exotoxin or a cytotoxic fragment thereof.

8. A composition of claim 7, wherein said *Pseudomonas* exotoxin is selected from the group consisting of PE35, PE38, PE38KDEL, PE40, PE4E, and PE38QQR.

9. A composition of claim 8, wherein the *Pseudomonas* exotoxin is PE38.

10. A composition of claim 3, further comprising a pharmaceutically acceptable carrier.

53

11. A composition of claim 4, further comprising a pharmaceutically acceptable carrier.
12. A composition of claim 5, further comprising a pharmaceutically acceptable carrier.
13. A composition of claim 6, further comprising a pharmaceutically acceptable carrier.
14. A composition of claim 7, further comprising a pharmaceutically acceptable carrier.
15. A composition of claim 8, further comprising a pharmaceutically acceptable carrier.
16. A composition of claim 9, further comprising a pharmaceutically acceptable carrier.
17. A method of inhibiting growth of a CD22+ cancer cell by contacting said cell with an anti-CD22 antibody with a variable light (V_L) chain having the sequence of a V_L of antibody RFB4 and a variable heavy (V_H) chain having the sequence a V_H chain of antibody RFB4, provided that residues 100, 100A and 100B of CDR3 of the V_H chain of said anti-CD22 antibody, as the residues are numbered in the column of FIG. 3 according to "Kabat Numbering System", have an amino acid sequence selected from the group consisting of: THW, YNW, TTW, and STY, which antibody is fused or conjugated to a therapeutic moiety, which therapeutic moiety inhibits growth of said cell.
18. A method of claim 17, wherein said antibody is selected from the group consisting of an scFv, a dsFv, a Fab, or a $F(ab')_2$.
19. A method of claim 17, wherein said therapeutic moiety is selected from the group consisting of a cytotoxin, a drug, a radioisotope, or a liposome loaded with a drug or a cytotoxin.
20. A method of claim 19, wherein the therapeutic moiety is a cytotoxin.
21. A method of claim 19, wherein the cytotoxin is selected from the group consisting of ricin A, abrin, ribotoxin, ribonuclease, saporin, calicheamycin, diphtheria toxin or a cytotoxic subunit or mutant thereof, a *Pseudomonas* exotoxin, a cytotoxic portion thereof, a mutated *Pseudomonas* exotoxin, a cytotoxic portion thereof, and botulinum toxins A through F.
22. A method of claim 21, wherein said cytotoxin is a *Pseudomonas* exotoxin or cytotoxic fragment thereof, or a mutated *Pseudomonas* exotoxin or a cytotoxic fragment thereof.

54

23. A method of claim 22, wherein said *Pseudomonas* exotoxin is selected from the group consisting of PE35, PE38, PE38KDEL, PE40, PE4E, and PE38QQR.
24. A method of claim 23, wherein the *Pseudomonas* exotoxin is PE38.
25. A method for detecting the presence of a CD22+ cancer cell in a biological sample, said method comprising:
- (a) contacting cells of said biological sample with an anti-CD22 antibody with a variable light (V_L) chain having the sequence of a V_L chain of antibody RFB4 and a variable heavy (V_H) chain having the sequence of a V_H chain of antibody RFB4, provided that residues 100, 100A and 100B of CDR3 of the V_H chain of said anti-CD22 antibody, as the residues are numbered in the column of FIG. 3 according to "Kabat Numbering System", have an amino acid sequence selected from the group consisting of: THW, YNW, TTW, and STY, said antibody being fused or conjugated to a detectable label; and,
 - (b) detecting the presence or absence of said label, wherein detecting the presence of said label indicates the presence of a CD22+ cancer cell in said sample.
26. A method of claim 25, wherein said antibody is selected from the group consisting of an scFv, a dsFv, a Fab, or a $F(ab')_2$.
27. A kit for detecting the presence of a CD22+ cancer cell in a biological sample, said kit comprising:
- (a) a container, and
 - (b) an anti-CD22 antibody with a variable light (V_L) chain having the sequence of a V_L chain of antibody RFB4 and a variable heavy (V_H) chain having the sequence of a V_H chain of antibody RFB4, provided that residues 100, 100A and 100B of CDR3 of the V_H chain of said anti-CD22 antibody, as the residues are numbered in the column of FIG. 3 according to "Kabat Numbering System", have an amino acid sequence selected from the group consisting of: THW, YNW, TTW, and STY, which antibody is fused or conjugated to a detectable label.
28. A kit of claim 27, wherein said antibody is selected from the group consisting of an scFv, a dsFv, a Fab, or a $F(ab')_2$.

* * * * *

-continued

Leu Glu Glu Lys Lys Gly Asn Tyr Val Val Thr Asp His Ser Gly Gly
 1 5 10 15

Xaa

<210> SEQ ID NO 8
 <211> LENGTH: 9
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence:
 Original phage amino acid, positions 95-100c

<400> SEQUENCE: 8

Gly Tyr Ser Ser Thr Ser Tyr Ala Met
 1 5

<210> SEQ ID NO 9
 <211> LENGTH: 9
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence:
 Original phage amino acid, positions 89-97

<400> SEQUENCE: 9

Leu Gln ser Phe Asn Val Pro Leu Thr
 1 5

<210> SEQ ID NO 10
 <211> LENGTH: 27
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence:
 Original phage amino acid, positions 95-100c

<400> SEQUENCE: 10

ggctattcta gtacctctta tgctatg

27

<210> SEQ ID NO 11
 <211> LENGTH: 27
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence:
 Nucleic acid sequence relating to original phage amino
 acid, positions 89-97

<400> SEQUENCE: 11

ttgcaaaagt ttaacgtgcc tcttaca

27

What is claimed is:

1. An isolated polypeptide comprising an antibody heavy chain variable region ("VH") and an antibody light chain variable region ("VL"), each region comprising three complementarity determining regions ("CDRs"), which CDRs of each region are numbered sequentially CDR1 to CDR3 starting from the amino terminus, the polypeptide when made into an immunotoxin with a *Pseudomonas* exotoxin A or cytotoxic fragment thereof ("PE") forming an immunotoxin which binds to epidermal growth factor receptor type III ("EGFRvIII") antigen and which has a cytotoxicity to cells expressing said antigen at least equal to the cytotoxicity to said cells of an immunotoxin of parental

antibody MR1 (SEQ ID NO.:18) and said PE, and a higher yield, when made into an immunotoxin with said PE, than that of MR1 when made into an immunotoxin with said PE, wherein:

- (a) CDRs 1-3, respectively of the V_H of the polypeptide have the sequence of CDRs 1-3, respectively of parental antibody MR1 V_H , except for:
 - (i) substitution of an amino acid selected from the group consisting of proline and tryptophan for the serine at position 98 of the CDR3 of the heavy chain variable region of antibody MR1, and
 - (ii) substitution of an amino acid selected from the group consisting of: tyrosine, asparagine, tryp-

tophan, isoleucine, phenylalanine, serine, and valine for the threonine at position 99 of the CDR3 of the heavy chain variable region of antibody MR 1 and, optionally,

- (iii) a substitution in CDR1 or CDR2 of said heavy chain variable region of at least one amino acid encoded by a codon that comprises a nucleotide belonging to a hot spot motif selected from AGY or RGYW, wherein R is A or G, Y is C or T and W is A or T; and

(b) CDRs 1-3, respectively, of the V_L of the polypeptide have:

- (i) the sequence of CDRs 1-3, respectively, of antibody MR1 V_L or,

- (ii) the sequence of CDRs 1-3, respectively, of antibody MR1 V_L except for a substitution in one or more of said CDRs 1-3 of said polypeptide at least one amino acid encoded by a codon that comprises a nucleotide belonging to a hot spot motif selected from AGY or RGYW, wherein R is A or G, Y is C or T and W is A or T.

2. The polypeptide of claim 1, further comprising a substitution in CDR1 or CDR2 of said heavy chain variable region of at least one amino acid, the amino acid encoded by a codon that comprises a nucleotide belonging to a hot spot motif selected from AGY or RGYW, wherein R is A or G, Y is C or T and W is A or T.

3. The polypeptide of claim 1, further comprising a substitution in a CDR of said antibody light chain variable region of at least one amino acid, the amino acid encoded by a codon that comprises a nucleotide belonging to a hot spot motif selected from AGY or RGYW, wherein R is A or G, Y is C or T and W is A or T.

4. The polypeptide of claim 1, wherein the substitution occurs in CDR1 or CDR2 of said light chain variable region.

5. The polypeptide of claim 1, wherein said substitutions in said V_H are selected from the group consisting of: S98P-T99Y, S98P-T99N, S98P-T99W, S98P-T99I, S98P-T99F, S98P-T99S, S98W-T99F, and S98P-T99V.

6. The polypeptide of claim 1, wherein said polypeptide is a scFv.

7. The polypeptide of claim 1, wherein said polypeptide is a dsFv, a Fab, or a F(ab')₂.

8. The polypeptide of claim 3, wherein the substitutions in the heavy chain variable region are S98P-T99Y and the substitution in the light chain variable region is F92W (antibody MR1-1).

9. A chimeric molecule comprising a polypeptide of claim 1, attached to an effector molecule, therapeutic moiety or a detectable label.

10. The chimeric molecule of claim 9, wherein the therapeutic moiety is a toxic moiety.

11. The chimeric molecule of claim 10, wherein the toxic moiety is a *Pseudomonas* exotoxin A ("PE") or a cytotoxic fragment thereof.

12. The chimeric molecule of claim 10, wherein the toxic moiety is a cytotoxic fragment, which is PE38.

13. The chimeric molecule of claim 11, wherein said chimeric molecule has an IC₅₀ of 7 ng/ml or lower.

14. The chimeric molecule of claim 11, wherein said chimeric molecule has an IC₅₀ of 5 ng/ml or lower.

15. The chimeric molecule of claim 11, wherein said chimeric molecule has an IC₅₀ of about 3.5 ng/ml.

16. The polypeptide chimeric molecule of claim 11, wherein said substitution is wherein said substitutions in said V_H are selected from the group consisting of: S98P-

T99Y, S98P-T99N, S98P-T99W, S98P-T99I, S98P-T99F, S98P-T99S, S98W-T99F, and S98P-T99V.

17. The chimeric molecule of claim 11, wherein said chimeric molecule has a yield of about 7%.

18. The chimeric molecule of claim 10, wherein the toxic moiety is selected from the group consisting of diphtheria toxin or a cytotoxic fragment thereof, saporin or a cytotoxic fragment thereof, pokeweed antiviral toxin or a cytotoxic fragment thereof, ricin or a cytotoxic fragment thereof, and bryodin 1 or a cytotoxic fragment thereof.

19. The chimeric molecule of claim 11, wherein the substitutions in the heavy chain are S98P-T99Y and further comprising a substitution in the light chain variable region of F92W (antibody MR1-1).

20. The polypeptide of claim 1, fused to a filamentous phage pIII protein.

21. A method of killing a cell bearing epidermal growth factor receptor type III ("EGFRvIII"), comprising contacting the cell with an immunotoxin comprising a toxic moiety and a targeting moiety, the targeting moiety comprising a polypeptide comprising an antibody heavy chain variable region (" V_H ") and an antibody light chain variable region (" V_L "), each region comprising three complementarity determining regions ("CDRs"), which CDRs of each region are numbered sequentially CDR1 to CDR3 starting from the amino terminus, the polypeptide, when made into an immunotoxin with a *Pseudomonas* exotoxin A or cytotoxic fragment thereof ("PE") forming an immunotoxin which binds to epidermal growth factor receptor type III ("EGFRvIII") antigen and which has a cytotoxicity to cells expressing said antigen at least equal to the cytotoxicity to said cells of an immunotoxin consisting of parental antibody MR1 (SEQ ID NO.:18) when made into an immunotoxin with said PE, and a higher yield, when made into an immunotoxin with said PE, than that of parental antibody MR1 (SEQ ID NO.:18) when made into an immunotoxin with said PE, wherein:

- (a) CDRs 1-3, respectively, of the V_H of the polypeptide have the sequence of CDRs 1-3, respectively, of parental antibody MR1 (SEQ ID NO.:18) V_H except for:

- (i) substitution of an amino acid selected from the group consisting of proline and tryptophan for the serine at position 98 of the CDR3 of the heavy chain variable region of antibody MR1, and

- (ii) substitution of an amino acid selected from the group consisting of: tyrosine, asparagine, tryptophan, isoleucine, phenylalanine, serine, and valine for the threonine at position 99 of the CDR3 of the heavy chain variable region of antibody MR1 and, optionally,

- (iii) a substitution in CDR1 or CDR2 of said heavy chain variable region of at least one amino acid encoded by a codon that comprises a nucleotide belonging to a hot spot motif selected from AGY or RGYW, wherein R is A or G, Y is C or T and W is A or T; and

- (b) CDRs 1-3, respectively, of the V_L of the polypeptide have:

- (i) the sequence of CDRs 1-3, respectively, of antibody MR1 V_L or,

- (ii) the sequence of CDRs 1-3, respectively, of antibody MR1 V_L except for a substitution in one or more of said CDRs 1-3 of said polypeptide at least one amino acid encoded by a codon that comprises a nucleotide belonging to a hot spot motif selected from AGY or RGYW, wherein R is A or G, Y is C or T and W is A or T.

49

22. The method of claim 21, wherein said polypeptide further comprises a substitution in CDR1 or CDR2 of said heavy chain variable region of at least one amino acid, the amino acid encoded by a codon that comprises a nucleotide belonging to a hot spot motif selected from AGY or RGYW, wherein R is A or G, Y is C or T and W is A or T.

23. The method of claim 21, wherein said antibody further comprises a substitution in a CDR of said light chain variable region of at least one amino acid, the amino acid encoded by a codon that comprises a nucleotide belonging to a hot spot motif selected from AGY or RGYW, wherein R is A or G, Y is C or T and W is A or T.

24. The method of claim 23, wherein the CDR3 mutated light chain variable region comprises a tryptophan substituted for a phenylalanine at position 92.

50

25. The method of claim 21, wherein the targeting moiety is antibody MR1-1.

26. The method of claim 21, wherein the cell is a malignant cell.

27. The method of claim 21, wherein the malignant cell is a glioma cell.

28. The method of claim 21, wherein the malignant cell is a breast carcinoma cell.

29. The method of claim 21, wherein the malignant cell is a lung carcinoma cell.

30. The method of claim 21, wherein the malignant cell is a ovarian carcinoma cell.

* * * * *

49

What is claimed is:

1. A vector comprising a nucleic acid sequence encoding a chimeric fusion protein comprising an IL-13 or circularly permuted IL-13 attached to a cytotoxic polypeptide.
2. The vector of claim 1, wherein said nucleic acid sequence encodes an IL-13-PE fusion protein.
3. The vector of claim 1, wherein said nucleic acid sequence encodes a cpIL-13-PE fusion protein.
4. The vector of claim 1, wherein said nucleic acid sequence encodes a fusion protein selected from the group consisting of IL-13-PE38QQR, cpIL-13-PE38QQR, IL-13-PE4E, and cpIL-13-PE4E.
5. The vector of claim 1, wherein the IL-13 is attached to the cytotoxic polypeptide through a linker.
6. A host cell comprising a nucleic acid sequence encoding a chimeric fusion protein comprising an IL-13 or circularly permuted IL-13 attached to a cytotoxic polypeptide.
7. The host cell of claim 6, wherein said nucleic acid sequence encodes an IL-13-PE fusion protein.
8. The host cell of claim 7, wherein said nucleic acid sequence encodes a fusion protein selected from the group consisting of IL-13-PE38QQR, cpIL-13-PE38QQR, IL-13-PE4E, and cpIL-13-PE4E.
9. The host cell of claim 6, wherein the IL-13 or the cpIL-13 is attached to the cytotoxic polypeptide through a linker.

50

10. The host cell of claim 6, wherein said nucleic acid sequence encodes a cpIL-13-PE fusion protein.

11. A chimeric molecule that specifically binds a tumor cell bearing an IL-13 receptor, said chimeric molecule comprising a cytotoxic molecule attached to a circularly permuted (cp) IL-13 that specifically binds an IL-13 receptor, which cpIL-13 is circularly permuted from an IL-13 having an amino terminus and a carboxy terminus, and further wherein said amino terminus and said carboxy terminus are joined through a peptide linker to form said cpIL-13.

12. A composition comprising a pharmaceutically acceptable carrier and a chimeric molecule, said chimeric molecule comprising:

an effector molecule attached to a circularly permuted ("cp") IL-13 that specifically binds to an IL-13 receptor, which cpIL-13 is circularly permuted from an IL-13 having an amino terminus and a carboxy terminus, and further wherein said amino terminus and said carboxy terminus are joined through a peptide linker to form said cpIL-13.

* * * * *

-continued

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
 (A) NAME/KEY: Protein
 (B) LOCATION: 1..112
 (D) OTHER INFORMATION: /note= "Human IgM GM607 VL region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

```

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1           5           10           15
Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
          20           25           30
Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gln Gln Ser
          35           40           45
Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
          50           55           60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
          65           70           75           80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Gly
          85           90           95
Leu Gln Thr Pro Gln Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
          100          105          110

```

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 112 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
 (A) NAME/KEY: Protein
 (B) LOCATION: 1..112
 (D) OTHER INFORMATION: /note= "Humanized B3 VL region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

```

Asp Val Leu Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1           5           10           15
Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ile Ile Val His Ser
          20           25           30
Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
          35           40           45
Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
          50           55           60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
          65           70           75           80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
          85           90           95
Ser His Val Pro Phe Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
          100          105          110

```

What is claimed is:

1. A method of inhibiting the growth of a cell bearing a Lewis^x antigen, said method comprising contacting said cell with a composition in an amount sufficient to inhibit the growth of said cell, said composition comprising:

- (a) a humanized Fv region of a light chain of an antibody and a humanized Fv region of a heavy chain of the same or of a different antibody, wherein said same or

different antibody is a monoclonal antibody selected from the group consisting of B1 (ATCC accession number HB 10572), B3 (ATCC accession number HB 10573), and B5 (ATCC accession number HB 10569), provided that

- (i) if the light chain and the heavy chain are both from antibody B3, the light chain and the heavy chain are independently selected from the group consisting of:

73

- a light chain with a leucine residue in position 4 and a heavy chain with an arginine residue in position 82b,
- (ii) if the heavy chain and the light chain are from antibody B1, a serine is substituted at position 95 of the heavy chain, and
- (iii) if the heavy chain and the light chain are from antibody B5, a serine is substituted at position 95 of the heavy chain; and
- (b) an effector molecule selected from the group consisting of a drug, a cytotoxin, a radioisotope, and a liposome loaded with a chemotherapeutic agent or a toxin; wherein said Fv regions and said effector molecule are joined to form a single molecule that has the binding specificity of a monoclonal antibody selected from the group consisting of B1, B3, and B5, thereby inhibiting the growth of said cell.
2. The method of claim 1, wherein said Fv region of a light chain and said Fv region of a heavy chain are from monoclonal antibody B3, wherein said light chain has a leucine residue in position 4.
3. The method of claim 1, wherein said Fv region of a light chain and said Fv region of a heavy chain are from monoclonal antibody B3, wherein said heavy chain has an arginine residue in position 82b.
4. The method of claim 1, wherein said effector molecule is a cytotoxin selected from the group consisting of a Pseudomonas exotoxin or cytotoxic fragment thereof, a Diphtheria toxin or cytotoxic fragment thereof, ricin or a cytotoxic fragment thereof, and abrin.
5. The method of claim 4, wherein said Pseudomonas exotoxin or cytotoxic fragment thereof is PE38.

74

6. The method of claim 1, wherein said drug is selected from the group consisting of vinblastin, daunomycin, and doxorubicin.
7. The method of claim 1, wherein said heavy chain is from monoclonal antibody B3 and said light chain is from monoclonal antibody B5.
8. The method of claim 1, wherein the cell is in a patient.
9. The method of claim 1, wherein said composition further comprises a pharmaceutically acceptable carrier.
10. The method of claim 1, wherein said Fv region of said light chain and said Fv region of said heavy chain are recombinantly fused to a cytotoxin.
11. The method of claim 10, wherein said cytotoxin is selected from the group consisting of Pseudomonas exotoxin or cytotoxic fragment thereof, a Diphtheria toxin or cytotoxic fragment thereof, ricin or a cytotoxic fragment thereof, and abrin.
12. The method of claim 11, wherein said heavy chain is from monoclonal antibody B3 and said light chain is from monoclonal antibody B5.
13. The method of claim 10, wherein said Fv region of a light chain and said Fv region of a heavy chain are from monoclonal antibody B3, wherein said light chain has a leucine residue in position 4.
14. The method of claim 10, wherein said Fv region of a light chain and said Fv region of a heavy chain are from monoclonal antibody B3, wherein said heavy chain has an arginine residue in position 82b.
15. The method of claim 10, wherein the cell is in a patient.
16. The method of claim 10, wherein said composition further comprises a pharmaceutically acceptable carrier.

* * * * *

TABLE 1-continued

Sequence Of Anti-Tac(Fv)-PE40	
1050	1080
ACCGGCAACGACGAGCGCGCGGCCAACGCCGACGTGGTGAGCCTGACCTGCCGGTC	
ThrGlyAsnAspGluAlaGlyAlaAlaAsnAlaAspValValSerLeuThrCysProVal	
1110	1140
GCCGCGGTGAATGCGCGGCGCGCGGACAGCGCGACGCCCTGCTGGAGCGCAACTAT	
AlaAlaGlyGluCysAlaGlyProAlaAspSerGlyAspAlaLeuLeuGluArgAsnTyr	
1170	1200
CCCACTGGCGCGAGTTCCTCGGCGACGCGGCCACGTCAGCTTCAGCACCCGCGGCAGC	
ProThrGlyAlaGluPheLeuGlyAspGlyGlyAspValSerPheSerThrArgGlyThr	
1230	1260
CAGAACTGGACGGTGGAGCGGCTGCTCCAGGCGCACCCCAACTGGAGGAGCGCGGTAT	
GlnAsnTrpThrValGluArgLeuLeuGlnAlaHisArgGlnLeuGluGluArgGlyTyr	
1290	1320
GTGTTTCGTCGGCTACACGGCACCTTCCTCGAAGCGCGCAAAGCATCGTCTTCGGCGGG	
ValPheValGlyTyrHisGlyThrPheLeuGluAlaAlaGlnSerIleValPheGlyGly	
1350	1380
GTGCGCGCGCGCAGCGACCTCGACGCGATCTGGCGCGTTTCTATATCGCCGGCGAT	
ValArgAlaArgSerGlnAspLeuAspAlaIleTrpArgGlyPheTyrIleAlaGlyAsp	
1410	1440
CCGGCGCTGGCTACGGCTACGCCAGGACCCAGGAACCCGACGCGCGCGCGGATCCGC	
ProAlaLeuAlaTyrGlyTyrAlaGlnAspGlnGluProAspAlaArgGlyArgIleArg	
1470	1500
AACGGTGCCCTGCTGCGGGTCTATGTGCGCGCTCGAGCCTGCCGGGCTTCTACCGCAC	
AsnGlyAlaLeuLeuArgValTyrValProArgSerSerLeuProGlyPheTyrArgThr	
1530	1560
AGCCTGACCTGGCGCGCGCGGAGCGCGCGGCGAGGTGCAACGGCTGATCGGCCATCCG	
SerLeuThrLeuAlaAlaProGluAlaAlaGlyGluValGluArgLeuIleGlyHisPro	
1590	1620
CTGCCGCTGCGCCTGGACGCCATCACCAGCGCCCGAGGAGGAAGCGGCGCCTGGAGACC	
LeuProLeuArgLeuAspAlaIleThrGlyProGluGluGluGlyGlyArgLeuGluThr	
1650	1680
ATTCTCGGTGGCGCTGGCCGAGCGCACCGTGGTGATTCCCTCGGCGATCCCCACCGAC	
IleLeuGlyTrpProLeuAlaGluArgThrValValIleProSerAlaIleProThrAsp	
1710	1740
CCGCGCAACGTGGCGCGGACCTCGACCCGTCAGCATCCCGACAAGGAACAGGCGATC	
ProArgAsnValGlyGlyAspLeuAspProSerSerIleProAspLysGluGlnAlaIle	
1770	
AGCGCCCTGCCGACTACGCCAGCCAGCCCGCAAACCGCCGCGGAGGACCTGAAG	
SerAlaLeuProAspTyrAlaSerGlnProGlyLysProProArgGluAspLeuLys	

The arrows respectively separate the V_H, linker, V_L, and PE40 regions

TABLE 2

CYTOTOXICITY (ID ₅₀) OF ANTI-TAC(Fv)-PE40 ON VARIOUS CELL LINES		
Cell Line	Anti-Tac(Fv)-PE40 ng/ml	PE ng/ml
HUT-102	0.15	10
OVCAR-3	>1000	30
KB	>1000	60
A431	>1000	4
CEM	>1000	200
Cr.IL2	2.7	Not done

Cell lines OVCAR3, KB and A431 were seeded at 1×10⁵/ml in 24-well plates one day prior to the addition of toxin. HUT-102 and CEM were washed twice and seeded at 3×10⁵/ml in 24 well plates (also see FIG. 3). Various

50 dilutions of toxin preparations were added, and 20 hours later the cells were labeled for 1.5 hr with ³H-leucine. The radioactivity in the TCA precipitate of the cells was determined. ID₅₀ is the concentration of toxin that inhibits protein synthesis by 50% as compared to a control with no toxin added. All the assays were done in duplicate and repeated three times.

What is claimed is:

60 1. A construct comprising DNA segments which direct the synthesis of a recombinant fusion protein in a suitable expression vector, wherein said fusion protein is a scFv-PE40.

2. A procaryotic or eucaryotic cell transformed or transfected with the construct of claim 1.

3. The construct of claim 1, wherein said construct directs the synthesis of a single chain polypeptide.

*

13

4. A procaryotic or eucaryotic cell transfected or transformed with the construct of claim 3.

5. The construct of claim 3, wherein said scFv is anti-Tac(Fv).

6. The construct of claim 5, wherein said DNA segments are contained in a plasmid.

7. The construct of claim 6 comprising plasmid of ATCC No. 67913.

14

8. The construct of claim 5, wherein said construct directs the synthesis of a fusion protein that is more cytotoxic than identical non-recombinant protein.

9. The construct of claim 5, wherein coding sequences for heavy chain variable domain is followed by coding sequences for light chain variable domain which is followed by the PE40 gene.

* * * * *

X

-continued

Asp Thr Thr Glu Lys Glu Thr Phe
1 5

(2) INFORMATION FOR SEQ ID NO:70:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION: 1..36
(D) OTHER INFORMATION: /note= "BK11B primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

TAAGAAGGAC ATATGCATAA GAACACAACCT GAGAAG

36

(2) INFORMATION FOR SEQ ID NO:71:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

Met His Lys Cys Asp
1 5

(2) INFORMATION FOR SEQ ID NO:72:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

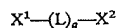
(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

Met His Lys Asn Thr Thr
1 5

What is claimed is:

1. A circularly permuted ligand selected from the group consisting of interleukin 4 (IL-4), interleukin 2 (IL-2), granulocyte colony stimulating factor (G-CSF), and granulocyte/macrophage colony stimulating factor (GM-CSF) that is a modification of an original ligand having amino acid residues numbered sequentially 1 through J with an amino terminus at residue 1 and a carboxyl terminus at residue J, said modified ligand having the formula shown below:



in which:

a is 0 or 1;

X¹ is a peptide consisting of an amino acid sequence having the sequence of residues n+1 through J of said original ligand;

X² is a peptide consisting of an amino acid sequence having the sequence of residues 1 through n of said original ligand;

n is an integer ranging from 1 to J-1; and

L is a linker; and further wherein the amino acid terminus of the modified ligand is located in X¹ and the carboxyl terminus of the modified ligand is located in X².

2. The circularly permuted ligand of claim 1, in which a is 1 and L is a peptide.

3. The circularly permuted ligand of claim 2, in which L is GGNGG (SEQ ID NO:50).

4. The circularly permuted ligand of claim 1, wherein said original ligand is interleukin 4 (IL-4).

5. The circularly permuted ligand of claim 4, wherein the first asparagine in the amino terminus of said original ligand is changed to an aspartate.

6. The circularly permuted ligand of claim 1, wherein said original ligand is interleukin 2 (IL-2).

7. The circularly permuted ligand of claim 1, wherein said original ligand is G-CSF.

8. The circularly permuted ligand of claim 1, wherein said original ligand is GM-CSF.

9. The circularly permuted ligand of claim 1, in which:

X¹ comprises the amino acid sequence having methionine followed by residues 38 through 129 of SEQ ID NO:2 (interleukin 4); and

X² comprises the amino acid sequence having residues 1 through 37 of SEQ ID NO:2 (interleukin 4).

10. The circularly permuted ligand of claim 9, in which a is 1 and L is the peptide GGNGG (SEQ ID NO:50).

11. The circularly permuted ligand of claim 1, in which: X¹ comprises the amino acid sequence having methionine followed by residues 105 through 129 of SEQ ID NO:2 (interleukin 4); and

X² comprises the amino acid sequence having residues 1 through 104 of SEQ ID NO:2 (interleukin 4).

12. The circularly permuted ligand of claim 11, in which a is 1 and L is the peptide GGNGG (SEQ ID NO:50).

13. The circularly permuted ligand of claim 1, in which: X¹ comprises the amino acid sequence having residues 39 through 134 of SEQ ID NO:3 (interleukin 2); and

X² comprises the amino acid sequence having residues 1 through 38 of SEQ ID NO:3 (interleukin 2).

14. The circularly permuted ligand of claim 13, in which a is 1 and L is the peptide GGNGG (SEQ ID NO: 50).

15. The circularly permuted ligand of claim 1, in which: X¹ comprises the amino acid sequence having residues 69 through 175 of SEQ ID NO:5 (G-CSF); and

X² comprises the amino acid sequence having residues 1 through 68 of SEQ ID NO:5 (G-CSF).

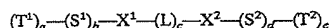
16. The circularly permuted ligand of claim 15 in which a is 1 and L is the peptide GGGNGGG (SEQ ID NO.52).

17. The circularly permuted ligand of claim 1, in which: X¹ comprises the amino acid sequence having residues 36 through 128 of SEQ ID NO:4 (GM-CSF); and

X² comprises the amino acid sequence having residues 1 through 36 of SEQ ID NO:4 (GM-CSF).

18. The circularly permuted ligand of claim 17 in which a is 1 and L is the peptide GGGNGGG (SEQ ID NO. 52).

19. A chimeric molecule comprising a circularly permuted interleukin ligand component that is a modification of an original interleukin ligand having amino acid residues numbered sequentially 1 through J with an amino terminus at residue 1 and a carboxyl terminus at residue J, said chimeric molecule having the following formula:



in which:

X¹ is a peptide consisting of an amino acid sequence having the sequence of residues n+1 through J of said original interleukin ligand;

L is a linker;

X² is a peptide consisting of an amino acid sequence having the sequence of residues 1 through n of said original interleukin ligand;

S¹ and S² are peptide spacers;

n is an integer ranging from 1 to J-1;

b, c and d are each independently 0 or 1;

a and e are either 0 or 1, provided that a and e cannot both be 0; and

T¹ and T² are proteins.

20. The chimeric molecule of claim 19, wherein said chimeric molecule is a fusion protein.

21. The chimeric molecule of claim 20, in which

a is zero;

b is zero;

c is 1;

d is 1;

e is 1; and

T² is a Pseudomonas exotoxin (PE) in which domain Ia is lacking.

22. The chimeric molecule of claim 21, in which: X¹ comprises methionine followed by the amino acid sequence having residues 38 through 129 of SEQ ID NO:2 (interleukin 4);

L is GGNGG (SEQ ID NO:50);

X² comprises the amino acid sequence having residues 1 through 37 of SEQ ID NO:2 (interleukin 4);

S² is ASGGPE (SEQ ID NO:57); and

T² is selected from the group consisting of PE38Q and PE38KDEL.

23. The chimeric molecule of claim 21, in which: X¹ comprises the amino acid methionine followed by the amino acid sequence having residues 105 through 129 of SEQ ID NO:2 (interleukin 4);

L is GGNGG (SEQ ID NO:50);

X² comprises the amino acid sequence having residues 1 through 104 of SEQ ID NO:2 (interleukin 4);

S² is SGGPE (SEQ ID NO:51); and

T² is selected from the group consisting of PE38Q and PE38KDEL.

24. The chimeric molecule of claim 21, in which: X¹ comprises amino acid methionine followed by the amino acid sequence having residues 39 through 134 of SEQ ID NO:3 (interleukin 2);

L is GGNGG (SEQ ID NO:50);

X² comprises the amino acid sequence having residues 1 through 38 of SEQ ID NO:3 (interleukin 2);

S² is SGGPE (SEQ ID NO:51); and

T² is selected from the group consisting of PE38Q and PE38KDEL.

25. The chimeric molecule of claim 20, in which:

a is 1;

b is 1;

c is 1;

d is zero;

e is zero; and

T¹ is a truncated Diphtheria toxin (DT).

26. The chimeric molecule of claim 25 in which: X¹ comprises the amino acid methionine followed by the amino acid sequence having residues 38 through 129 of SEQ ID NO:2 (interleukin 4);

L is GGNGG (SEQ ID NO:50);

X² comprises the amino acid sequence having residues 1 through 37 of SEQ ID NO:2 (interleukin 4);

S¹ is HM; and

T¹ is DT388.

27. The chimeric molecule of claim 25, in which: X¹ comprises the amino acid methionine followed by the amino acid sequence having residues 105 through 129 of SEQ ID NO:2 (interleukin 4);

L is GGNGG (SEQ ID NO:50);

X² comprises the amino acid sequence having residues 1 through 104 of SEQ ID NO:2 (interleukin 4);

S¹ is RPHMAD (SEQ ID NO:53); and

T¹ is DT388.

28. The chimeric molecule of claim 20, in which:

a is 1;

b is 1;

d is 0;

e is 0; and

T¹ is an antibody.

29. The chimeric molecule of claim 28, in which:

X¹ comprises the amino acid methionine followed by the amino acid sequence corresponding to residues 38 through 129 of SEQ ID NO:3 (interleukin 4);

L is GGNGG (SEQ ID NO:50);

X² comprises the amino acid sequence having residues 1 through 37 of SEQ ID NO:2 (interleukin 4);

S¹ is ASGGPE (SEQ ID NO:57); and

T¹ is B3(Fv).

30. The chimeric molecule of claim 28, in which:

X¹ comprises the amino acid methionine followed by the amino acid sequence having residues 105 through 129 of SEQ ID NO:3 (interleukin 4);

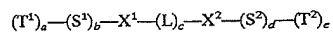
L is GGNGG (SEQ ID NO:50);

X² comprises the amino acid sequence having residues 1 through 104 of SEQ ID NO:2 (interleukin 4);

S¹ is SGGPE (SEQ ID NO:51); and

T¹ is B3(Fv).

31. A method of inhibiting growth of tumor cells in an organism, said method comprising contacting said cells with a composition comprising a fusion protein said fusion protein comprising a modified ligand component selected from the group consisting of cytokines and colony stimulating factors that is a modification of an original ligand selected from the group consisting of cytokines and colony stimulating factors that specifically binds a tumor cell, said original ligand having amino acid residues numbered sequentially 1 through J with an amino terminus at residue 1 and a carboxyl terminus at residue J, the fusion protein having the following formula:



in which:

X¹ is a peptide consisting of an amino acid sequence having the sequence of residues n+1 through J of said original ligand;

L is a linker

X² is a peptide consisting of an amino acid sequence having the sequence of residues 1 through n of said original ligand;

S¹ and S² are peptide spacers;

n is an integer ranging from 1 to J-1; and

b, c, and d are each independently 0 or 1;

a and e are either 0 or 1, provided that a and e cannot both be 0; and

T¹ and T² are cytotoxins.

32. The method of claim 31, wherein said original ligand is interleukin 4 (IL-4).

33. The method of claim 32, in which:

X¹ is methionine followed by the amino acid sequence having residues 38 through 129 of SEQ ID NO:2 (interleukin 4);

L is GGNGG (SEQ ID NO:50);

X² is the amino acid sequence having residues 1 through 37 of SEQ ID NO:2 (interleukin 4);

S² is ASGGPE (SEQ ID NO:57); and

T² is selected from the group consisting of PE38Q and PE38KDEL.

34. The method of claim 32, in which:

X¹ is the amino acid methionine followed by the amino acid sequence having residues 105 through 129 of SEQ ID NO:2 (interleukin 4);

L is GGNGG (SEQ ID NO:50);

X² comprises the amino acid sequence corresponding to residues 1 through 104 of SEQ ID NO:2 (interleukin 4);

S² is SGGPE (SEQ ID NO:51); and

T² is selected from the group consisting of PE38Q and PE38KDEL.

35. The method of claim 31, wherein said original ligand is interleukin 2 (IL-2).

36. The method of claim 31, wherein said original ligand is granulocyte colony stimulating factor (G-CSF).

37. The method of claim 31, wherein said original ligand is granulocyte/macrophage colony stimulating factor (GM-CSF).

38. The method of claim 31, in which

a is zero;

b is zero;

c is 1;

d is 1;

e is 1; and

T² is a Pseudomonas exotoxin in which domain Ia is lacking.

39. The method of claim 31, in which:

a is 1;

b is 1;

c is 1;

d is zero;

e is zero; and

T¹ is a truncated Diphtheria toxin.

40. The method of claim 31 in which:

X¹ is the amino acid methionine followed by the amino acid sequence having residues 38 through 129 of SEQ ID NO:2 (interleukin 4);

L is GGNGG (SEQ ID NO:50);

X² the amino acid sequence having residues 1 through 37 of SEQ ID NO:2 (interleukin 4);

S¹ is HM; and

T¹ is DT388.

41. The method of claim 31, in which:

X¹ is the amino acid methionine followed by the amino acid sequence having residues 105 through 129 of SEQ ID NO:2 (interleukin 4);

L is GGNGG (SEQ ID NO:50);

X² is the amino acid sequence having residues 1 through 104 of SEQ ID NO:2 (interleukin 4);

S¹ is RPHMAD (SEQ ID NO:53); and

T¹ is DT388.

42. A method of specifically delivering a first molecule to a target cell in vivo said method comprising:

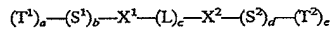
administering to a mammal a molecule comprising at circularly permuted ligand selected from the group consisting of cytokines and colony stimulating factors in a pharmaceutically acceptable carrier; wherein said ligand specifically binds said target cell.

43. The method of claim 42, wherein said ligand is selected from the group consisting of interleukin 2 (IL-2), interleukin 4 (IL-4), granulocyte/macrophage colony stimu-

lating factor (GM-CSF), and granulocyte colony stimulating factor (G-CSF).

44. The method of claim 43, wherein said ligand is attached to a cytotoxin.

45. A chimeric molecule comprising a circularly permuted growth factor ligand component that is a modification of an original growth factor ligand having amino acid residues numbered sequentially 1 through J with an amino terminus at residue 1 and a carboxyl terminus at residue J, said chimeric molecule having the following formula,



in which:

X¹ is a peptide consisting of an amino acid sequence having the sequence of residues n+1 through J of said original growth factor ligand;

L is a linker;

X² is a peptide consisting of an amino acid sequence having the sequence of residues 1 through n of said original growth factor ligand;

S¹ and S² are peptide spacers;

n is an integer ranging from 1 to J-1;

b, c and d are each independently 0 or 1;

a and e are either 0 or 1, provided that a and e cannot both be 0; and

T¹ and T² are proteins.

46. The chimeric molecule of claim 45, wherein said chimeric molecule is a fusion protein.

47. The chimeric molecule of claim 46, in which

a is zero;

b is zero;

c is 1;

d is 1;

e is 1; and

T² is a *Pseudomonas* exotoxin (PE) in which the domain Ia is lacking.

48. The chimeric molecule of claim 47, in which:

X¹ comprises the amino acid methionine followed by the amino acid sequence having residues 36 through 128 of SEQ ID NO:4 (granulocyte/macrophage colony stimulating factor (GM-CSF);

L is GGGNGGG (SEQ ID NO:52);

X² comprises the amino acid sequence having residues 1 through 35 of SEQ ID NO:4 (GM-CSF);

S² is SGGPE (SEQ ID NO:51); and

T² is selected from the group consisting of PE38Q and PE38KDEL.

49. The chimeric molecule of claim 47, in which:

X¹ comprises the amino acid methionine followed by the amino acid sequence having residues 69 through 175 of SEQ ID NO:5 (granulocyte colony stimulating factor (G-CSF));

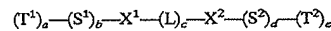
L is GGGNGGG (SEQ ID NO:52);

X² comprises the amino acid sequence having residues 1 through 68 of SEQ ID NO:5 (G-CSF);

S² is SGGPE (SEQ ID NO:51); and

T² is selected from the group consisting of PE38Q and PE38KDEL.

50. A chimeric molecule comprising a circularly permuted cytokine ligand component that is a modification of an original cytokine ligand having amino acid residues numbered sequentially 1 through J with an amino terminus at residue 1 and a carboxyl terminus at residue J, said chimeric molecule having the following formula:



in which:

X¹ is a peptide consisting of an amino acid sequence having the sequence of residues n+1 through J of said original cytokine ligand;

L is a linker;

X² is a peptide consisting of an amino acid sequence having the sequence of residues 1 through n of said original cytokine ligand;

S¹ and S² are peptide spacers;

n is an integer ranging from 1 to J-1;

b, c and d are each independently 0 or 1;

a and e are either 0 or 1, provided that a and e cannot both be 0; and

T¹ and T² are proteins.

* * * * *

-continued

(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Gly Gly Gly Gly Ser
1 5

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Arg Glu Asp Leu Lys
1 5

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Arg Glu Asp Leu
1

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Arg Asp Glu Leu
1

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Lys Asp Glu Leu

What is claimed is:

1. A chimeric molecule that specifically binds a tumor cell bearing an IL-13 receptor, said chimeric molecule comprising a cytotoxin attached to a targeting molecule that is an

IL-13 molecule or an antibody that specifically binds an IL-13 receptor.

2. The chimeric molecule of claim 1, wherein said targeting molecule is human IL-13.

27

3. The chimeric molecule of claim 1, wherein said cytotoxin is selected from the group consisting of Pseudomonas exotoxin, ricin, abrin and Diphtheria toxin.

4. The chimeric molecule of claim 3, wherein chimeric molecule is a single-chain fusion protein.

5. The chimeric molecule of claim 4, wherein said cytotoxin is a Pseudomonas exotoxin.

6. The chimeric molecule of claim 5, wherein said Pseudomonas exotoxin is PE38QQR.

7. A chimeric molecule that specifically binds a tumor cell bearing an IL-13 receptor, said chimeric molecule comprising an effector molecule attached to an antibody that specifically binds an IL-13 receptor,

wherein said effector molecule is selected from the group consisting of a cytotoxin, a label, a radionuclide and a liposome, wherein the liposome contains a cytotoxin, a label, or a radionuclide.

8. A composition comprising a pharmacologically acceptable carrier and a chimeric molecule that specifically binds a tumor cell bearing an IL-13 receptor, said chimeric molecule comprising:

an effector molecule attached to

a targeting molecule that is an IL-13 molecule or an antibody that specifically binds an IL-13 receptor,

wherein said effector molecule is selected from the group consisting of a cytotoxin, a label, a radionuclide and a

28

liposome, wherein the liposome contains a cytotoxin, a label, or a radionuclide.

9. The composition of claim 8, wherein said targeting molecule is IL-13.

10. The composition of claim 9, wherein chimeric molecule is a single-chain fusion protein.

11. The composition of claim 10, wherein said effector molecule is a Pseudomonas exotoxin.

12. The composition of claim 11, wherein said Pseudomonas exotoxin is PE38QQR.

13. A chimeric molecule that specifically binds a tumor cell bearing an IL-13 receptor, said chimeric molecule comprising an effector molecule attached to targeting molecule comprising an antibody that specifically binds an IL-13 receptor, wherein said effector molecule is linked to said targeting molecule by a linker consisting of a ligand.

14. A composition comprising a pharmacologically acceptable carrier and a chimeric molecule that specifically binds a tumor cell bearing an IL-13 receptor, said chimeric molecule comprising:

an effector molecule attached to

a targeting molecule that is an IL-13 molecule or an antibody that specifically binds an IL-13 receptor, wherein said effector molecule is linked to said targeting molecule by a linker consisting of a ligand.

* * * * *

-continued

50					55					60				
Lys 65	Gly	Arg	Phe	Thr	Ile 70	Ser	Arg	Asp	Asn 75	Ser	Lys	Asn	Thr	Leu 80
Leu	Gln	Met	Asn	Arg 85	Leu	Arg	Ala	Glu	Asp 90	Thr	Ala	Ile	Tyr	Ser 95
Ala	Arg	Gly	Leu 100	Ala	Trp	Gly	Ala	Trp 105	Phe	Ala	Tyr	Trp	Gly 110	Gln 115
Thr	Leu	Val	Thr	Val	Ser	Ser								

What is claimed is:

1. A recombinant DNA molecule that encodes a single chain fusion protein, said recombinant DNA molecule comprising:

i) a DNA sequence that encodes a humanized Fv region of both the light and the heavy chains of a B3 antibody (ATCC Accession No. HB 10573) wherein said heavy chain has a serine at position 95 as shown in SEQ ID NO:47; and

ii) a DNA sequence that encodes an effector molecule; wherein said DNA sequences are recombinantly fused to form a single molecule.

2. The recombinant DNA molecule of claim 1, wherein said DNA sequence encodes an Fv region comprising a humanized variable light chain having the amino acid sequence designated SEQ ID NO:50.

3. The recombinant DNA molecule of claim 1, wherein said DNA sequence encodes an Fv region comprising a humanized variable heavy chain having the amino acid sequence designated SEQ ID NO:47.

4. The recombinant DNA molecule of claim 1, wherein said effector molecule is a *Pseudomonas* exotoxin.

5. The recombinant DNA molecule of claim 4, wherein said effector molecule is selected from the group consisting of PE38, PE40, PE38KDEL and PE38REDL.

6. A single chain fusion protein comprising:

a) a humanized Fv region of both the light and heavy chains of a B3 antibody (ATCC Accession No. HB 10573) wherein said heavy chain has a serine at position 95 as shown in SEQ ID NO:47; and

b) an effector molecule wherein the humanized Fv region and the effector molecule are recombinantly fused to form a single molecule.

7. The fusion protein of claim 6, wherein the Fv region of said protein comprises a humanized variable light chain having the amino acid sequence designated SEQ ID NO:50.

8. The fusion protein of claim 6, wherein said protein comprises a humanized variable heavy chain having the amino acid sequence designated SEQ ID NO:47.

9. The fusion protein of claim 6, wherein said effector molecule is a *Pseudomonas* exotoxin.

10. The fusion protein of claim 9, wherein said *Pseudomonas* exotoxin is PE38, PE40, PE38KDEL and PE38REDL.

11. A recombinant DNA molecule that encodes a humanized Fv region of both the light and heavy chains of a B3 monoclonal antibody (ATCC Accession No. HB 10573) and wherein said heavy chain has a serine at position 95 as shown in SEQ ID NO:47.

12. The recombinant DNA molecule of claim 11, wherein said DNA sequence encodes an Fv region comprising a humanized variable light chain having the amino acid sequence designated SEQ ID NO:50.

13. The recombinant DNA molecule of claim 11, wherein said DNA sequence encodes an Fv region comprising a humanized variable heavy chain having the amino acid sequence designated SEQ ID NO:47.

14. A recombinantly produced protein comprising a humanized Fv region of both a light and a heavy chain of a B3 monoclonal antibody (ATCC Accession No. HB10573) and wherein said heavy chain has a serine at position 95 as shown in SEQ ID NO:47.

15. The protein of claim 14, wherein said protein comprises a humanized variable light chain having the amino acid sequence designated SEQ ID NO:50.

16. The protein of claim 14, wherein said protein comprises a humanized variable heavy chain having the amino acid sequence designated SEQ ID NO:47.

17. A pharmaceutical composition comprising a recombinantly produced single chain fusion protein in a concentration sufficient to inhibit tumor growth, together with a pharmaceutically acceptable carrier wherein said fusion protein comprises:

a) a humanized Fv region of both a light and a heavy chain of a B3 monoclonal antibody (ATCC Accession No. HB10573) wherein said heavy chain has a serine at position 95 as shown in SEQ ID NO:47; and

b) an effector molecule; wherein both of said Fv regions and said effector molecule are recombinantly fused to form a single molecule that has the binding specificity of the B3 monoclonal antibody (ATCC Accession No. HB10573).

18. The composition of claim 17, wherein said effector molecule is a *Pseudomonas* exotoxin.

19. The composition of claim 18, wherein said effector molecule is selected from the group consisting of PE38, PE40, PE38KDEL, PE38REDL.

20. The composition of claim 17, wherein said humanized Fv region is a humanized B3(Fv) region.

21. A method of detecting the presence or absence of a cell bearing a Lewis^x carbohydrate antigen in a patient, said method comprising the steps of:

a) removing a tissue or fluid sample from said patient;

b) adding an antibody to said sample wherein said antibody comprises:

i) a humanized Fv region of both a light and a heavy chain of a B3 monoclonal antibody (ATCC Accession No. HB10573) wherein said heavy chain has a serine at position 95 as shown in SEQ ID NO:47; and

ii) an effector molecule; further wherein said Fv regions are recombinantly fused to form a single molecule that has the binding specificity of the B3 monoclonal antibody; and

c) detecting for the presence or absence of a binding complex between the antibody and the antigen.

* * * * *

TABLE 2-continued

CYTOTOXICITY (ID ₅₀) OF ANTI-TAC(Fv)-PE40 ON VARIOUS CELL LINES		
Cell Line	Anti-Tac(Fv)-PE40 ng/ml	PE ng/ml
CEM	>1000	200
Cr.II.2	2.7	Not done

Cell lines OVCAR3, KB and A431 were seeded at 1×10^5 /ml in 24-well plates one day prior to the addition of toxin. HUT-102 and CEM were washed twice and seeded at 3×10^5 /ml in 24 well plates (also see FIG. 3). Various dilutions of toxin preparations were added, and 20 hours later the cells were labeled for 1.5 hr with ^3H -leucine. The radioactivity in the TCA precipitate of the cells was determined. ID₅₀ is the concentration of toxin that inhibits protein

synthesis by 50% as compared to a control with no toxin added. All the assays were done in duplicate and repeated three times.

What is claimed is:

- 5 1. A method for achieving targeted cytotoxicity, comprising contacting cells targeted to be killed with a cytotoxic amount of an antibody-PE40 recombinant fusion protein, wherein said antibody is a single-chain Fv fragment (scFv) and said PE40 is a *Pseudomonas* exotoxin (PE) fragment 10 omitting amino acids 1 through 252 and possessing at least the translocating and ADP ribosylating activity of PE, and wherein said cells targeted to be killed have receptors or antigens to which said antibody binds, a wherein said fusion protein has lower toxicity to cells which lack receptors or 15 antigens for the binding of said antibody.
2. The method of claim 1, wherein said fusion protein, is a single polypeptide chain.
3. The method of claim 1, wherein said antibody is anti-Tac(Fv).

* * * * *

-continued

(i i) MOLECULE TYPE: peptide

(i i i) HYPOTHETICAL: NO

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:16:

L y s A s p G l u L e u
1

What is claimed is:

1. An isolated and purified recombinant *Pseudomonas* exotoxin (PE) fusion protein wherein a ligand binding agent is fused to a PE molecule in which domain Ia is deleted and from 1 to 28 amino acids from the amino terminal end of domain II are deleted.

2. The recombinant PE of claim 1, wherein the ligand binding agent is fused after about amino acid position 607 and is followed by amino acids 604-613 of domain III.

3. The recombinant PE of claim 1, wherein the ligand binding agent is TGF α .

4. The recombinant PE of claim 1, wherein the ligand binding agent is an antibody or binding fragment thereof.

5. The recombinant PE of claim 1, wherein the ligand binding agent is a hormone.

6. The recombinant PE of claim 1, wherein the ligand binding agent is a growth factor.

7. The recombinant PE of claim 1, wherein the ligand binding agent specifically binds a cancer cell receptor.

8. The recombinant PE of claim 1, comprising amino acids 280 to 364 and 381 to 613 of PE with TGF α inserted within the recombinant PE molecule after about amino acid 607 and followed by amino acids 604-613 of domain III.

9. The recombinant PE molecule of claim 1, wherein the PE molecule includes an endoplasmic retention sequence at a carboxyl terminal end of the molecule.

10. The recombinant PE fusion protein of claim 1, wherein the molecule further comprises a substantial deletion of domain III.

11. The recombinant fusion protein of claim 10, wherein about amino acids 604 to 613 of domain III are retained.

12. The recombinant PE fusion protein of claim 11, wherein the ligand binding agent is fused to the PE in place of deleted domain III.

13. An isolated and purified recombinant *Pseudomonas* exotoxin (PE) fusion protein wherein a ligand binding agent is fused to a PE molecule in which:

(a) domain Ia is deleted;

(b) from 1 to 28 amino acids from the amino terminal end of domain II are deleted; and,

(c) a methionine residue is inserted at the resultant amino terminus of said molecule;

(d) wherein the fusion protein is further characterized in that it is at least twenty times more cytotoxic to target cells bound by the ligand binding agent in a cytotoxicity assay when compared with an unmodified PE40 fused to the ligand binding agent.

* * * * *

-continued

(i i) MOLECULE TYPE: peptide

(i i i) HYPOTHETICAL: NO

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:16:

L y s A s p G l u L e u

1

What is claimed is:

1. A method for impairing tumor growth in a patient comprising administering to the patient intravenously, into a body cavity or into a lumen of an organ a ligand binding agent specific for a tumor cell, fused to a recombinant *Pseudomonas* exotoxin molecule in which:

- (a) domain Ia is deleted;
- (b) from 1 to 28 amino acids from the amino terminal end of domain II are deleted;
- (c) a methionine occurs at the resultant amino terminal of said molecule; and,
- (d) said molecule has increased toxic activity to a target cell as compared to an unmodified PE40.

2. The method of claim 1, wherein the recombinant *Pseudomonas* exotoxin molecule has amino acids 280 to 364 and 381 to 613 of Sequence ID No: 1 wherein residue 364 is peptide bonded to residue 381.

3. The method of claim 1, wherein the *Pseudomonas* exotoxin molecule includes a substitution of serine for the amino acid cysteine at position 287 of Sequence ID No: 1.

4. The method of claim 1, wherein the molecule further includes an amino acid sequence at a carboxyl terminal end

of the molecule selected from the group consisting of REDLK, REDL, and KDEL.

5. The method of claim 1, wherein amino acids 604-613 of domain III in Sequence ID No: 1 are retained in the *Pseudomonas* exotoxin molecule.

6. A method for impairing tumor growth in a patient comprising administering to the patient intravenously, into a body cavity or into a lumen of an organ a ligand binding agent specific for a tumor cell, fused to a recombinant *Pseudomonas* exotoxin molecule (PE) having a deletion in the amino terminal end of domain II such that the molecule is at least 20 times more cytotoxic to target cells than unmodified PE40 in a cytotoxicity assay wherein the cytotoxicity to the target cells of unmodified PE40 and the recombinant PE molecule is measured by assaying against the target cells (i) unmodified PE40 fused to a ligand binding agent specific for the target cells and (ii) the recombinant PE molecule fused to a ligand binding agent specific for the target cells.

* * * * *

I. ACTIVITY OF TGF α -PE40 AND KDEL DERIVATIVES (ID₅₀) ON CELLS WITH EGF RECEPTORS.

	A431 ng/ml	KB ng/ml	OVCAR3 ng/ml	HUT102 ng/ml
TGF α -PE40	.35	.96	5.4	>312
TGF α -PE40 KDEL*	.44			
	.048	.37	.84	>312
	.034			
TGF α -PE40 (KDEL) ₃	.076	.12	1.1	>312
	.022			

*TGF α -PE40 (253-609 KDEL)

**TGF α -PE40 (253-609 KDEL KDEL KDEL)

This table shows that replacing the last 5 amino acids of TGF α -PE40 with KDEL or (KDEL)₃ increases its activity 3 to 10-fold.

II. CYTOTOXICITY OF CD4-PE40 DERIVATIVES ON ENV-5 CELLS THAT EXPRESS gp120 OF HUMAN IMMUNODEFICIENCY VIRUS.

PROTEIN	ID ₅₀ (ng/ml)
CD4-PE40 · REDLK	2.5
CD4-PE40 · KDEL	0.5
CD4-PE40 · (KDEL) ₃	0.65

Increased cytotoxicity of CD4-PE40 on target cells expressing HIV gp120 produced by replacing the last 5 amino acids of CD4-PE40 with KDEL or (KDEL)₃. ENV-5 cells express gp120.

TABLE D

Comparison of cytotoxic activity of bifunctional and monofunctional recombinant fusion proteins.

	ID ₅₀ * (ng/ml)	
	HUT102	A431
TGF α -anti-Tac(Fv)-PE40	7.8	12.0
Anti-Tac(Fv)-PE40	2.3	~500
TGF α -PE40	>500	0.5

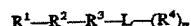
*ID₅₀ is the concentration of the fusion protein that gave 50% inhibition of protein synthesis.

What is claimed is:

1. A method for killing a target cell, said method comprising contacting said target cell with a cytotoxic amount of a composition comprising a recombinant *Pseudomonas* exotoxin (PE) having a first recognition molecule for binding said target cell and a carboxyl terminal sequence of 4 to 16 amino acids which permits translocation of the PE molecule into a cytosol of said target cell, the first recognition molecule being inserted in domain III after and no acid 600 and before amino acid 613 of the PE.

2. A method of killing targeted cells, said method comprising the step of contacting cells targeted to be killed, with a cytotoxic amount of a recombinant *Pseudomonas* exotoxin fusion protein containing at least two different recognition molecules for killing cells expressing receptors to which said recognition molecules specifically bind.

3. The method of claim 1, wherein said carboxyl terminal sequence comprises, in a direction from the amino terminus to the carboxyl terminus, the following amino acids:



wherein,

R¹ is a positively charged amino acid;
R² is a negatively charged amino acid;
R³ is a negatively charged amino acid;
R⁴ is a positively charged amino acid; and
n is zero or 1.

4. The method of claim 3, wherein R¹ is selected from the group consisting of R and K.

5. The method of claim 4, wherein R² is selected from the group consisting of E and D.

6. The method of claim 4, wherein R³ is selected from the group consisting of E and D.

7. The method of claim 4, wherein n is 1 and R⁵ is selected from the group consisting of K and R.

8. The method of claim 4, wherein the carboxy terminal sequence is selected from the group consisting of REDLK, KEDLK, REISLR, REDL, and KDEL.

9. The method of claim 4, wherein the carboxy terminal sequence is KDELKDELKDEL.

10. The method of claim 4, wherein the first recognition molecule is an antibody or a portion of an antibody which recognizes the target cell.

11. The method of claim 4, wherein the first recognition molecule is selected from the group consisting of a growth factor, lymphokine, cytokine, and a hormone.

12. The method of claim 4, wherein the first recognition molecule is TGF α or CD4.

13. The method of claim 4, wherein the first recognition molecule is inserted after amino acid 607 of the PE.

14. The method of claim 4, wherein a second recognition molecule is attached to the amino terminus of said *Pseudomonas* exotoxin.

15. The method of claim 14, wherein the second recognition molecule is different from the first recognition molecule.

16. The method of claim 14, wherein the second recognition molecule is anti-Tac(Fv).

17. The method of claim 14, wherein the recombinant PE is TGF α -anti-Tac(Fv)-PE40.

18. The method of claim 2, wherein said two different recognition molecules comprise a first recognition molecule inserted in the carboxyl terminus of said *Pseudomonas* exotoxin, and a second recognition molecule attached to the amino terminus of said *Pseudomonas* exotoxin.

19. The method of claim 18, wherein said first recognition molecule is inserted in domain III after amino acid 600 and before amino acid 613 of said *Pseudomonas* exotoxin.

20. The method of claim 19, wherein said first recognition molecule is inserted in domain III after amino acid 607 of said of said *Pseudomonas* exotoxin molecule.

21. The method of claim 19, wherein the first recognition molecule is TGF α or CD4.

* * * * *

-continued

(A) NAME/KEY: -
 (B) LOCATION: 1.60
 (D) OTHER INFORMATION: /note="VK76"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GAAGATGGAT CACAGTTGGT GAATTCATTA AGCTTTGAGC TCCAGCTTGG TCCCAGAACC 60

What is claimed is:

1. An antibody-PE40 recombinant fusion protein wherein said antibody is a single-chain Fv fragment (scFv) and said PE40 is a *Pseudomonas* exotoxin (PE) fragment omitting amino acids 1 through 252 possessing at least the translocating and ADP ribosylating activity of PE.

2. The fusion protein of claim 1, wherein said fusion protein has a single polypeptide chain.

3. The fusion protein of claim 2 wherein the antibody is anti-Tac(Fv).

10

4. A composition comprising an effective amount of the fusion protein of claim 1 to kill cells bearing a receptor or an antigen to which the antibody binds, and a pharmaceutically acceptable carrier.

15

5. The composition of claim 4, wherein the antibody is anti-Tac(Fv).

* * * * *

-continued

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Gly Gly Gly Gly Ser
1 5

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15
Gly Gly Gly Ser
20

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
1 5 10 15
Ser Gly Gly Gly Gly Ser
20

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

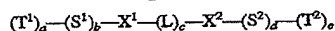
(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Ala Ser Gly Gly Pro Glu
1 5

What is claimed is:

1. A fusion protein comprising a modified interleukin 4 (IL4) that is a modification of an original IL4 having amino acid residues numbered sequentially 1 through J with an amino terminus at residue 1 and a carboxyl terminus at residue J, said fusion protein having the following formula:



in which:

X¹ is a peptide consisting of an amino acid sequence having the sequence of residues n+1 through J of said original IL4;

L is a linker;

X² is a peptide consisting of an amino acid sequence having the sequence of residues 1 through n of said original IL4;

S¹ and S² are peptide spacers;

n is an integer ranging from 1 to J-1;

b, c, and d are each independently 0 or 1;

a and e are each either 0 or 1, provided that a and e cannot both be 0; and

T¹ and T² are cytotoxins.

2. The fusion protein of claim 1, wherein said cytotoxin is a Pseudomonas exotoxin in which domain Ia is lacking or a Diphtheria toxin in which the native receptor-binding domain is removed by truncation of the Diphtheria toxin B chain.

3. The fusion protein of claim 2, in which:

a is zero;

b is zero;

c is 1;

d is 1;

e is 1; and

T² is a Pseudomonas exotoxin in which domain Ia is lacking.

4. The fusion protein of claim 3, in which:

X¹ consists of methionine followed by the amino acid sequence having the sequence of residues 38 through 129 of SEQ ID NO: 3 (IL4);

L is GGNGG (SEQ ID NO: 50);

X² consists of the amino acid sequence having the sequence of residues 1 through 37 of SEQ ID NO: 3 (IL4);

S² is SGGPE (SEQ ID NO: 51); and

T² is PE38QQRDEL.

5. The fusion protein of claim 3, in which:

X¹ consists of methionine followed by the amino acid sequence having the sequence of residues 105 through 129 of SEQ ID NO: 3 (IL4);

L is GGNGG (SEQ ID NO: 50);

X² consists of the amino acid sequence having the sequence of residues 1 through 104 of SEQ ID NO: 3 (IL4);

S² is SGGPE (SEQ ID NO: 51); and

T² is PE38QQRDEL.

6. The fusion protein of claim 2, in which:

a is 1;

b is 1;

c is 1;

d is zero;

e is zero; and

T¹ is a truncated Diphtheria toxin.

7. The fusion protein of claim 6, in which:

X¹ consists of methionine followed by the amino acid sequence having the sequence of residues 38 through 129 of SEQ ID NO: 3 (IL4);

L is GGNGG (SEQ ID NO: 50);

X² consists of the amino acid sequence having the sequence of residues 1 through 37 of SEQ ID NO: 3 (IL4);

S¹ is HM; and

T¹ is DT388.

8. The fusion protein of claim 6, in which:

X¹ consists of methionine followed by the amino acid sequence having the sequence of residues 105 through 129 of SEQ ID NO: 3 (IL4);

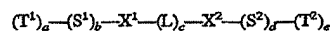
L is GGNGG (SEQ ID NO: 50);

X² consists of the amino acid sequence having the sequence of residues 1 through 104 of SEQ ID NO: 3 (IL4);

S¹ is RPHMAD (SEQ ID NO: 53); and

T¹ is DT388.

9. A fusion protein comprising a modified interleukin 2 (IL2) that is a modification of an original IL2 having amino acid residues numbered sequentially 1 through J with an amino terminus at residue 1 and a carboxyl terminus at residue J, said fusion protein having the following formula:



in which:

X¹ is a peptide consisting of an amino acid sequence having the sequence of residues n+1 through J of said original IL2;

L is a linker;

X² is a peptide consisting of an amino acid sequence having the sequence of residues 1 through n of said original IL2;

S¹ and S² are peptide spacers;

n is an integer ranging from 1 to J-1;

b, c, and d are each independently 0 or 1;

a and e are each either 0 or 1, provided that a and e cannot both be 0; and

T¹ and T² are cytotoxins.

10. The fusion protein of claim 9, wherein said cytotoxin is a Pseudomonas exotoxin in which domain Ia is lacking or a Diphtheria toxin in which the native receptor-binding domain is removed by truncation of the Diphtheria toxin B chain.

11. The fusion protein of claim 10, in which:

X¹ consists of methionine followed by the amino acid sequence having the sequence of residues 39 through 134 of SEQ ID NO: 4 (IL2);

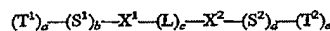
L is GGNGG (SEQ ID NO: 50);

X² consists of the amino acid sequence having the sequence of residues 1 through 38 of SEQ ID NO: 4 (IL2);

S² is SGGPE (SEQ ID NO: 51); and

T² is PE38QQRDEL.

12. A fusion protein comprising a modified granulocyte macrophage colony stimulating factor (GM-CSF) that is a modification of an original GM-CSF having amino acid residues numbered sequentially 1 through J with an amino terminus at residue 1 and a carboxyl terminus at residue J, said fusion protein having the following formula:



in which:

X¹ is a peptide consisting of an amino acid sequence having the sequence of residues n+1 through J of said original GM-CSF;

L is a linker;

X² is a peptide consisting of an amino acid sequence having the sequence of residues 1 through n of said original GM-CSF;

S¹ and S² are peptide spacers;

n is an integer ranging from 1 to J-1;

b, c, and d are each independently 0 or 1;

a and e are each either 0 or 1, provided that a and e cannot both be 0; and

T¹ and T² are cytotoxins.

13. The fusion protein of claim 12, wherein said cytotoxin is a Pseudomonas exotoxin in which domain Ia is lacking or a Diphtheria toxin in which the native receptor-binding domain is removed by truncation of the Diphtheria toxin B chain.

14. The fusion protein of claim 12, in which:

X¹ consists of methionine followed by the amino acid sequence having the sequence of residues 36 through 128 of SEQ ID NO: 5 (GM-CSF);

L is GGGNGGG (SEQ ID NO: 52);

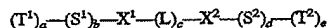
X² consists of the amino acid sequence having the sequence of residues 1 through 35 of SEQ ID NO: 5 (GM-CSF);

57

X S² is SGGPE (SEQ ID NO: 51); and

T² is PE38QQRDEL.

15. A fusion protein comprising a modified granulocyte colony stimulating factor (G-CSF) that is a modification of an original G-CSF having amino acid residues numbered sequentially 1 through J with an amino terminus at residue 1 and a carboxyl terminus at residue J, said fusion protein having the following formula:



in which:

X¹ is a peptide consisting of an amino acid sequence having the sequence of residues n+1 through J of said original G-CSF;

L is a linker;

X² is a peptide consisting of an amino acid sequence having the sequence of residues 1 through n of said original G-CSF;

S¹ and S² are peptide spacers;

n is an integer ranging from 1 to J-1;

b, c, and d are each independently 0 or 1;

58

a and e are each either 0 or 1, provided that a and e cannot both be 0; and

T¹ and T² are cytotoxins.

16. The fusion protein of claim 15, wherein said cytotoxin is a Pseudomonas exotoxin in which domain Ia is lacking or a Diphtheria toxin in which the native receptor-binding domain is removed by truncation of the Diphtheria toxin B chain.

17. The fusion protein of claim 16, in which:

X¹ consists of methionine followed by the amino acid sequence having the sequence of residues 69 through 175 of SEQ ID NO: 6 (G-CSF);

L is GGGNGGG (SEQ ID NO: 52);

X² consists of the amino acid sequence having the sequence of residues 1 through 68 of SEQ ID NO: 6 (G-CSF);

S² is SGGPE (SEQ ID NO: 51); and

T² is PE38QQRDEL.

* * * * *

possess hIL-13 binding sites and such cells are sensitive to hIL-13-PE38QQR chimeric toxin.

Because the hIL-13R has been suggested to share the λ_2 subunit of the IL-2R (Russell et al. *Science* 262: 1880-1883 (1993)), the specificity of hIL-13-PE38QQR action on A431 and CRL1739 cells, the two cell lines with different sensitivities to the chimeric toxin was further explored. The cells were treated with hIL-13-PE38QQR with or without rhIL-2 at a concentration of 1.0 $\mu\text{g/ml}$ or 10 $\mu\text{g/ml}$. The rhIL-2 did not have any blocking action on hIL-13-PE38QQR on the two cell lines, even at 10,000 fold molar excess over the chimeric toxin. These results indicate that the cell killing by the hIL-13-toxin is independent of the presence of hIL-2. IL-4, unlike IL-2, blocks the action of IL-13-PE38QQR.

Native hIL-4 was added to cells which were then treated with hIL-13-PE38QQR. Unexpectedly, it was found that hIL-4 inhibited the cytotoxic activity of the hIL-13-toxin. This phenomenon was seen on all the tested cell lines, including Colo201, A431 and CRL1739. To investigate the possibility that hIL-13 and hIL-4 may compete for the same binding site, the cells were also treated with the hIL-4-based recombinant toxin, hIL-4-PE38QQR (Debinski et al. *Int. J. Cancer* 8: 74-748 (1994)). The cytotoxic action of hIL-4-PE38QQR had already been shown to be blocked by an excess of hIL-4 but not of hIL-2 (Id.). In the present experiment hIL-13 potentially blocked the cytotoxic activity of hIL-4-PE38QQR. Also, the action of another hIL-4-based chimeric toxin, hIL-4-PE4E (Debinski et al. *J. Biol. Chem.* 268: 14065-14070 (1993)), was blocked by an excess of hIL-13 on Colo201 and A431 cells. Thus, the cytotoxicity of hIL-13-PE38QQR is blocked by an excess of hIL-13 or hIL-4, and the cytotoxic action of hIL-4-PE38QQR is also blocked by the same two growth factors. However, IL-2 does not block the action of either chimeric toxin. These results strongly suggest that hIL-4 and hIL-13 have affinities for a common binding site.

This conclusion was supported by the observation of one cytokine blocking the effect of a mixture of the two chimeric toxins. When A431 cells were incubated with both hIL-3- and hIL-4-PE38QQR chimeric toxins concomitantly the cytotoxic action was preserved and additive effect was observed as expected. An excess of hIL-13 efficiently blocked the action of a mixture of the two chimeric toxins. Moreover, neither hIL-13 nor hIL-4 blocked cell killing by another mixture composed of hIL-13-PE38QQR and TGF α -PE40, a chimeric toxin which targets the EGFR (TGF α -based chimeric toxin, TGF α -PE40) (Siegal et al. *EASEB J.* 3, 2647-2652 (1992)). The same was observed on Colo201 cells.

Reciprocal Blocking of Chimeric Toxins by IL-13 and IL-4 is due to competition for binding sites

The binding ability of human IL-13 was compared to human IL-4-PE38QQR in competitive binding assays. Recombinant hIL-4-PE38QQR was labeled with ^{125}I using the lactoperoxidase method as described by Debinski et al., *J. Clin. Invest.* 90, 405-411 (1992). Binding assays were performed by a standard saturation and displacement curves analysis. A431 epidermoid carcinoma cells were seeded at 10^5 cells per well in a 24-well tissue culture plates at 24 h before the experiment. The plates were placed on ice and cells were washed with ice-cold PBS without Ca^{++} , Mg^{++} in 0.2% BSA, as described (Id.). Increasing concentrations of hIL-13 or hIL-4-PE38QQR were added to cells and incubated 30 min prior to the addition of fixed amount of ^{125}I -hIL-4-PE38QQR (specific activity 6.2 $\mu\text{Ci}/\mu\text{g}$ protein) for 2 to 3 h. After incubation, the cells were washed twice and lysed with 0.1N NaOH, and the radioactivity was counted in a γ -counter.

Human IL-4-PE38QQR competed for the binding of ^{125}I -hIL-4-PE38QQR to A431 cells with an apparent ID_{50} of $4 \times 10^{-8} \text{M}$. In addition, hIL-13 also competed for the ^{125}I -hIL-4-PE38QQR binding site with a comparable potency to that exhibited by the chimeric protein. More extensive binding studies have shown that hIL-13 also competes for hIL-4 binding sites on human renal carcinoma cell lines.

The possibility of an influence of hIL-13 or hIL-4 on the process of receptor-mediated endocytosis and post-binding PE cellular toxicity steps was excluded by adding to cells: (i) native PE (PE binds to the α_2 -macroglobulin receptor), (ii) TGF α -PE40, and (iii) a recombinant immunotoxin C242rF(ab)-PE38QQR (Debinski et al. *Clin. Res.* 42, 251A, (Abstr.) (1994)). C242rF(ab)-PE38QQR binds a tumor-associated antigen that is a sialylated glycoprotein (Debinski et al. *J. Clin. Invest.* 90: 405-411 (1992)). The expected cytotoxic actions of these recombinant toxins were observed and neither hIL-13 nor hIL-4 blocked these actions on A431 and Colo205 cells.

hIL-4 and hIL-13 compete for a common binding site on carcinoma cell but evoke different biological effects

Even though hIL-13 and hIL-4 compete for a common binding site, they induce different cellular effects. Protein synthesis was inhibited in A431 epidermoid carcinoma cells in a dose-dependent manner by hIL-4 alone, or by a ADP-ribosylation deficient chimeric toxin containing hIL-4 (Debinski et al. *Int. J. Cancer* 58: 744-748 (1994)). This effect of hIL-4 or enzymatically deficient chimeric toxin can be best seen with a prolonged time of incubation (≥ 24 h) and requires concentrations of hIL-4 many fold higher than that of the active chimeric toxin in order to cause a substantial decrease in tritium incorporation. However, when A431 cells were treated with various concentrations of hIL-13, no inhibition (or stimulation) of protein synthesis was observed, even at concentrations as high as 10 $\mu\text{g/ml}$ of hIL-13 for a 72 h incubation. The same lack of response to hIL-13 was found on renal cell carcinoma cells PM-RCC. Thus, while hIL-13 and hIL-4 may possess a common binding site, they appear to transduce differently in carcinoma cells expressing this common site, such as A431 and PM-RCC cells.

The above examples are provided to illustrate the invention but not to limit its scope. Other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference.

What is claimed is:

1. A method for specifically delivering an effector molecule to a solid tumor cell bearing an IL-13 receptor, said method comprising:
 - a) providing a chimeric molecule comprising said effector molecule attached to a targeting molecule selected from the group consisting of an IL-13, and an anti-IL-13 receptor antibody; and
 - b) contacting said chimeric molecule with said tumor cell to specifically bind said chimeric molecule to said tumor cell.
2. The method of claim 1, wherein said targeting molecule is IL-13.
3. The method of claim 1, wherein said tumor is a carcinoma.
4. The method of claim 1, wherein said effector molecule is selected from the group consisting of a cytotoxin, a label, a radionuclide, a drug, a liposome, a ligand, and an antibody.
5. The method of claim 4, wherein said effector molecule is a *Pseudomonas* exotoxin.

25

6. The method of claim 5, wherein chimeric molecule is a fusion protein.

7. The method of claim 6, wherein said fusion protein is IL-13-PE38QQR.

8. The method of claim 1, wherein said effector molecule is a detectable label and said method further comprises detecting said label and thereby detecting said tumor cell.

9. The method of claim 8, wherein said detectable label is selected from the group consisting of a radiolabel, an enzyme, a colorimetric label, a fluorescent label, and a magnetic bead.

10. The method of claim 8, wherein said label is a fluorescent label.

11. The method of claim 8, wherein said detecting is by scintillography.

12. The method of claim 8, wherein said tumor cell is a carcinoma cell.

13. The method of claim 11, wherein said carcinoma is selected from the group consisting of colon carcinoma, skin carcinoma, and gastric carcinoma.

14. A method for impairing growth of a solid tumor cell bearing an IL-13 receptor, said method comprising contacting said tumor cell with a chimeric molecule comprising:

a targeting molecule selected from the group consisting of an IL-13, and an anti-IL-13 receptor antibody; and

26

an effector molecule selected from the group consisting of a Pseudomonas exotoxin, a Diphtheria toxin, and a radionuclide, wherein said effector molecule may be linked to the targeting molecule by a linker consisting of a ligand or an antibody; and wherein said contacting specifically binds said chimeric molecule to said tumor cell.

15. The method of claim 14, wherein said targeting molecule is a human IL-13.

16. The method of claim 15, wherein said effector molecule is a Pseudomonas exotoxin or a Diphtheria toxin.

17. The method of claim 16, wherein chimeric molecule is a single-chain fusion protein.

18. The method of claim 17, wherein said effector molecule is a Pseudomonas exotoxin.

19. The method of claim 18, wherein said Pseudomonas exotoxin is PE38QQR.

20. The method of claim 15, wherein said tumor cell growth is tumor cell growth in a human.

21. The method of claim 20, wherein said contacting comprises administering said chimeric molecule to the human intravenously, into a body cavity, or into a lumen or an organ.

* * * * *

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Glu	Val	Lys	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly
1				5					10					15

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 125 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:

(A) NAME/KEY: Region
 (B) LOCATION: 1..125
 (D) OTHER INFORMATION: /note="Mouse monoclonal antibody B5 Fv
 Light chain region"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Asp	Val	Leu	Leu	Thr	Gln	Thr	Pro	Leu	Ser	Leu	Pro	Val	Ser	Leu	Gly
1				5					10					15	
Asp	Gln	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Ile	Val	His	Ser
			20					25					30		
Asn	Gly	Asn	Thr	Tyr	Leu	Glu	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser
		35					40					45			
Pro	Lys	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	Ser	Gly	Val	Pro
	50					55				60					
Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile
65				70					75					80	
Ser	Arg	Val	Glu	Ala	Glu	Asp	Leu	Gly	Val	Tyr	Tyr	Cys	Phe	Gln	Gly
			85						90					95	
Ser	His	Val	Pro	Phe	Thr	Phe	Gly	Ser	Gly	Thr	Lys	Leu	Glu	Ile	Lys
			100					105					110		
Arg	Ala	Asp	Ala	Ala	Pro	Thr	Val	Ser	Ile	Phe	Pro	Pro			
		115					120					125			

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:68:

Asp	Val	Leu	Leu	Thr	Gln	Thr	Pro	Leu	Ser	Leu	Pro	Val	Ser	Leu
1				5					10					15

What is claimed is:

1. A recombinant DNA molecule that encodes a single 65 chain fusion protein, said recombinant DNA molecule comprising:

- a) a DNA sequence that encodes the Fv region of both the light and the heavy chains of an antibody; and
- b) a DNA sequence that encodes an effector molecule selected from the group consisting of a truncated

85

Pseudomonas exotoxin, and a detectable label, wherein said fusion protein specifically binds an epitope bound by monoclonal antibody B3 (ATCC Accession Number HB10569).

2. The recombinant DNA molecule of claim 1, wherein said effector molecule is a *Pseudomonas* exotoxin.

3. The recombinant DNA molecule of claim 2, wherein said effector molecule is selected from the group consisting of PE38, PE40, PE38KDEL, and PE38REDL.

4. The recombinant DNA molecule of claim 1, wherein said antibody is B3.

5. The recombinant DNA molecule of claim 1, wherein said molecule encodes a fusion protein selected from the group consisting of B3(Fv)-PE38, B3(Fv)-PE40, B3(Fv)-PE38KDEL, and B3(Fv)-PE38REDL.

6. A recombinantly produced single chain fusion protein comprising:

- a) the Fv region of both the light and heavy chains of an antibody; and
- b) an effector molecule; wherein said Fv region and said effector molecule are recombinantly fused to form a single chain molecule that has the binding specificity of monoclonal antibody B3.

7. The fusion protein of claim 6, wherein said effector molecule is a *Pseudomonas* exotoxin.

8. The fusion protein of claim 7, wherein said effector molecule is selected from the group consisting of PE38, PE40, PE38KDEL and PE38REDL.

9. The fusion protein of claim 6, wherein said antibody is B3.

10. The fusion protein of claim 6, wherein said fusion protein is selected from the group consisting of B3(Fv)-PE38, B3(Fv)-PE40, B3(Fv)-PE38KDEL, and B3(Fv)-PE38REDL.

86

11. A recombinant DNA molecule that encodes a single chain antibody, said recombinant DNA molecule comprising a DNA sequence that encodes the Fv region of both the light and heavy chains of an antibody; wherein said DNA sequences are recombinantly fused to form a single molecule and wherein said fusion protein has the binding specificity of monoclonal antibody B3.

12. The recombinant DNA molecule of claim 11, wherein said antibody is B3.

13. A recombinantly produced single chain antibody comprising an Fv region of both a light and a heavy chain of an antibody where said light and heavy chains are recombinantly fused to form a single molecule which has the binding specificity of monoclonal antibody B3.

14. The single chain antibody of claim 13, wherein said antibody is B3.

15. A pharmaceutical composition comprising a recombinantly produced single chain fusion protein in a concentration sufficient to inhibit tumor cell growth, together with a pharmaceutically acceptable carrier wherein said fusion protein comprises:

- a) a single-chain Fv region of an antibody, said Fv region comprising the V_H and V_L regions of said antibody; and
- b) an effector molecule; wherein said Fv region and said effector molecule are recombinantly fused to form a single molecule that has the binding specificity of monoclonal antibody B3.

16. The composition of claim 15, wherein said effector molecule is a *Pseudomonas* exotoxin.

17. The composition of claim 16, wherein said effector molecule is selected from the group consisting of PE38, PE40, PE38KDEL, and PE38REDL.

18. The composition of claim 17 wherein said antibody is B3.

* * * * *

-continued

(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```

Met Trp Glu Gln Leu Glu Gln Ser Gln Tyr Pro Val Gln Arg
1           5           10

```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```

Met Trp Glu Gln Leu Glu Gln
1           5

```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```

Arg Glu Asp Leu Lys
1           5

```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```

Arg Glu Asp Leu
1

```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```

Lys Asp Glu Leu
1

```

What is claimed is:

1. An isolated and purified recombinant *Pseudomonas* exotoxin (PE) molecule having a deletion in the amino terminal end of domain II such that the molecule is at least twenty times more cytotoxic to target cells than unmodified

PE40 in a cytotoxicity assay wherein the cytotoxicity to the target cells of unmodified PE40 and the recombinant PE molecule is measured by assaying against the target cells (i) PE40 fused to a ligand binding agent specific for the target

✱

cells and (ii) the recombinant PE molecule fused to a ligand binding agent specific-for the target cells.

2. The recombinant PE of claim 1, wherein the molecule has amino acids 280 to 364 and 381 to 613 of Sequence ID NO: 1 wherein residue 364 is peptide bonded to residue 381. 5

3. The recombinant PE of claim 1, wherein the molecule includes a substitution of serine for the amino acid cysteine at position 287 of Sequence ID No: 1.

4. The recombinant PE of claim 1, wherein the molecule further includes an amino acid sequence at a carboxyl terminal end of the molecule selected from the group consisting of REDLK, REDL, and KDEL. 10

5. The recombinant PE of claim 1, wherein the molecule further comprises a deletion in domain III.

6. The recombinant PE of claim 5, wherein amino acids 15 604-613 of domain III in Sequence ID No: 1 are retained.

7. An isolated and purified recombinant *Pseudomonas* exotoxin (PE), molecule in which:

(a) domain Ia is deleted;

(b) from 1 to 28 amino acids from the amino terminal end 20 of domain II are deleted;

(c) a methionine at the resultant amino terminal of said molecule; and,

(d) said molecule has increased toxic activity to a target cell as compared to an unmodified PF40.

8. The recombinant PE of claim 7, wherein the molecule has amino acids 280 to 364 and 381 to 613 of Sequence ID NO: 1 wherein residue 364 is peptide bonded to residue 381.

9. The recombinant PE of claim 7, wherein the molecule includes a substitution of serine for the amino acid cysteine at position 287 of Sequence ID No: 1.

10. The recombinant PE of claim 7, wherein the molecule further includes an amino acid sequence at a carboxyl terminal end of the molecule selected from the group consisting of REDLK, REDL, and KDEL.

11. The recombinant PE of claim 7, wherein the molecule further comprises a deletion in domain III.

12. The recombinant PE of claim 11, wherein amino acids 604-613 of domain III in Sequence ID No: 1 are retained.

13. A pharmaceutical composition comprising the molecule of claim 7 and a pharmaceutically acceptable carrier.

* * * * *

TABLE IX

LD ₅₀ ANALYSIS		
Molecule	Amount Injected	# Deaths/# mice
IL6-PE40	5 µg	0/4
	10 µg	0/4
	15 µg	0/2
	20 µg	2/4
	25 µg	3/4
IL6-II-PE40	50 µg	2/2
	5 µg	0/2
	10 µg	0/2
	20 µg	1/2
IL6-PE66 ^{4Glu}	30 µg	2/2
	5 µg	0/2
	10 µg	1/2
	20 µg	2/2

Mice were administered a single dose, I.P. with indicated amounts of IL6-toxin and the number of dead mice were determined after 72 hours.

TABLE X

Molecule	Size	Amount Injected	Peak	Detection Limit	Maximum Detected
IL6-PE40	60 kD	15 µg	1 hr	8 hr	5 µg/ml
IL6-II-PE40	72 kD	15 µg	1 hr	8 hr	6 µg/ml
IL6-PE66 ^{4Glu}	86 kD	15 µg	1 hr	8 hr	12 µg/ml

Mice were injected I.P. with a single dose and serum levels of the chimeric toxin were determined at 5 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr and 24 hr by assaying cytotoxic activity on U266 cells. The levels at 8 hr was approximately 0.5 µg/ml

TABLE XI

ACTIVITY OF TGFα-PE-4Glu and CD4(178)PE-4Glu on TARGET CELLS	
	ID ₅₀ (ng/ml)
TGFαPE ^{4Glu}	0.007 ^a
CD4(178)PE ^{4Glu}	1.5 ^b

^aon A431 cells in a 20 hr assay.

^bon CV-1 cells expressing gp120, in a 4 hr assay.

What is claimed is:

1. A recombinant mutant *Pseudomonas* exotoxin (PE) having a positively charged amino acid residue in domain Ia substituted by an amino acid residue without a positive charge, so that the mutant PE has a lower animal toxicity compared to the unsubstituted molecule, said mutant PE being selected from the group consisting of a PE in which amino acids 57, 246, 247 and 249 are glutamic acid (PE-Glu- 57,246,247,249), a PE in which amino acid 57 is a glutamic acid and amino acids 241-250 are deleted (PE-Glu-57Δ241-250) and a PE in which amino acid 57 is a glutamic acid and amino acids 246, 247, and 249 are glycine (PE-Glu- 57-Gly246,247,249).

2. A recombinant mutant *Pseudomonas* exotoxin (PE) attached to a targeting agent which recognizes a specific site on a cell targeted to be killed selected from the group consisting of IL6 attached to a PE in which amino acids 57, 246, 247 and 249 are glutamic acid (IL6-PE 66-4-Glu), an IL6 attached to a PE in which amino acid 57 is glutamic acid and amino acids 246, 247 and 249 are glycine (IL6-PE-Glu-57Gly246,247,249), a TGFα attached to a PE in which amino acids 57, 246, 247 and 249 are glutamic acid (TGFα-PE66-4Glu) and CD4 attached to a PE in which amino acid 57 is glutamic acid and amino acids 246, 247 and 249 are glycine (CD4-PE66-4Glu).

3. The PE of claim 2 being IL6-PE66-4Glu.

4. The PE of claim 2 being IL6-PEGlu57Gly246,247,249.

5. The PE of claim 2 being TGFα-PE66-4Glu.

6. The PE of claim 2 being CD4-PE66-4Glu.

7. A composition comprising a cytotoxic amount of the PE of claim 2 and a pharmaceutically acceptable carrier.

8. A recombinant mutant *Pseudomonas* exotoxin (PE) comprising IL6-domainII-PE40.

9. A composition comprising a cytotoxic amount of the recombinant mutant *Pseudomonas* exotoxin (PE) of claim 8 to kill cells bearing IL6 receptors, and a pharmaceutically acceptable carrier.

* * * * *

13

TABLE B

Cytotoxic activity on Swiss 3T3 cells of various PE derivatives		
Plasmid	Protein ^a	ID ₅₀ (ng/ml)
pVC 45f+T	PE 1-608 REDLK	1.6
pVC 49415f+T	PE 1-608 KDEL	0.76
pSS 49445f+T	PE 1-608 KDELKDELKDEL	0.55

^aPE proteins were purified on Mono Q column and were approximately 90% pure.

^bSame as Table A.

TABLE C

I. ACTIVITY OF TGFα-PE40 AND KDEL DERIVATIVES (ID ₅₀) ON CELLS WITH EFG RECEPTORS.				
	A431 ng/ml	KB ng/ml	OVCAR 3 ng/ml	HUT 102 ng/ml
TGFα-PE40	.35 .44	.96	5.4	>312
TGFα-PE40 KDEL*	.048 .034	.37	.84	>312
TGFα-PE40 (KDEL) ₃ **	.076 .022	.12	1.1	>312

*TGFα-PE40 (253-609 KDEL)

**TGFα-PE40 (253-609 KDEL KDEL KDEL)

This TABLE shows that replacing the last 5 amino acids of TGFα-PE40 with KDEL or (KDEL)₃ increases its activity 3 to 10-fold.

II. CYTOTOXICITY OF CD4-PE40 DERIVATIVES ON ENV-5 CELLS THAT EXPRESS gp120 OF HUMAN IMMUNODEFICIENCY VIRUS.

PROTEIN	ID ₅₀ (ng/ml)
CD4-PE40+REDLK	2.5
CD4-PE40+KDEL	0.5
CD4-PE40+(KDEL) ₃	0.65

Increased cytotoxicity of CD4-PE40 on target cells expressing HIV gp120 produced by replacing the last 5 amino acids of CD4-PE40 with KDEL or (KDEL)₃. ENV-5 cells express gp120.

TABLE D

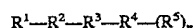
Comparison of cytotoxic activity of bifunctional and monofunctional recombinant fusion proteins.		
	ID ₅₀ ^a (ng/ml)	
	HUT102	A431
TGFα-anti-Tac(Fv)-PE40	7.8	12.0
Anti-Tac(Fv)-PE40	2.3	~500
TGFα-PE40	>500	0.5

^aID₅₀ is the concentration of the fusion protein that gave 50% inhibition of protein synthesis.

What is claimed is:

1. A fusion protein comprising a recombinant *Pseudomonas* exotoxin (PE) molecule, a first recognition moiety for binding a target cell, and a carboxyl terminal sequence of 4 to 16 residues which permits translocation of said fusion protein into the target cell cytosol, the first recognition moiety being inserted in domain III of PE after residue 600 and before residue 613.

2. The fusion protein of claim 1, wherein the carboxyl terminal sequence comprises, in a direction from the amino terminus to the carboxyl terminus, the following amino acid residues:



wherein,

14

R¹ is a positively charged amino acid residue;

R² is a negatively charged amino acid residue;

R³ is a negatively charged amino acid residue;

R⁴ is L; and

R⁵ is a positively charged amino acid residue;

and wherein n is zero or 1.

3. The fusion protein of claim 2, wherein R¹ is selected from the group consisting of R and K.

4. The fusion protein of claim 2, wherein R² is selected from the group consisting of E and D.

5. The fusion protein of claim 2, wherein R³ is selected from the group consisting of D and E.

6. The fusion protein of claim 2, wherein n is 1 and R⁵ is selected from the group consisting of K and R.

7. The fusion protein of claim 2, wherein the carboxyl terminal sequence is selected from the group consisting of REDLK, KEDLK, REDLR, REDL, and KDEL.

8. The fusion protein of claim 2, wherein the carboxyl terminal sequence is KDELKDELKDEL.

9. The fusion protein of claim 2, wherein the first recognition molecule is an antibody or a portion of an antibody which recognizes the target cell.

10. The fusion protein of claim 2, wherein the first recognition molecule is selected from the group consisting of a growth factor, lymphokine, cytokine, and a hormone.

11. The fusion protein of claim 2, wherein the first recognition molecule is TGFα or CD4.

12. The fusion protein of claim 2, wherein the first recognition molecule is inserted after residue 607 of the PE molecule.

13. The fusion protein of claim 2, wherein a second recognition molecule is inserted in the toxin molecule.

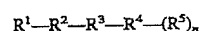
14. The fusion protein of claim 13, wherein the second recognition molecule is different from the first recognition molecule.

15. The fusion protein of claim 13, wherein the second recognition molecule is anti-Tac (Fv).

16. The fusion protein of claim 13, wherein the recombinant PE molecule is TGFα-anti-Tac(Fv)-PE40.

17. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a fusion protein comprising a recombinant *Pseudomonas* exotoxin (PE) molecule, a first recognition moiety for binding a target cell, and a carboxyl terminal sequence of 4 to 16 residues which permits translocation of said fusion protein into the target cell cytosol, the first recognition moiety being inserted in domain III of PE after residue 600 and before residue 613.

18. The composition of claim 17, wherein the carboxyl terminal sequence comprises, in a direction from the amino terminus to the carboxyl terminus, the following amino acid residues:



wherein,

R¹ is a positively charged amino acid residue;

R² is a negatively charged amino acid residue;

R³ is a negatively charged amino acid residue;

R⁴ is L; and

R⁵ is a positively charged amino acid residue; and wherein n is zero or 1.

19. The composition of claim 17, wherein the carboxyl terminal residues are selected from the group consisting of REDLK, KEDLK, REDLR, REDL, and KDEL.

20. The fusion protein of claim 7 wherein the carboxyl terminal sequence is REDLK.

15

21. The fusion protein of claim 7 wherein the carboxyl terminal sequence is KEDLK.

22. The fusion protein of claim 7 wherein the carboxyl terminal sequence is REDLR.

23. The fusion protein of claim 7 wherein the carboxyl terminal sequence is REDL.

24. The fusion protein of claim 7 wherein the carboxyl terminal sequence is KDEL.

25. The fusion protein of claim 19 wherein the carboxyl terminal sequence is REDLK.

16

26. The composition of claim 19 wherein the carboxyl terminal sequence is KEDLK.

27. The composition of claim 19 wherein the carboxyl terminal sequence is REDLR.

28. The composition of claim 19 wherein the carboxyl terminal sequence is REDL.

29. The fusion protein of claim 19 wherein the carboxyl terminal sequence is KDEL.

* * * * *

kill HIV-infected cells in a pharmaceutically acceptable vehicle, if necessary, such as physiological saline, buffered solutions and the like. The toxin may be administered by any suitable route, systemically or locally as deemed more effective. The method of controlling or treating AIDS comprises contacting HIV-infected cells with the effective amount of the recombinant toxin [CD4(178)-PE40 fusion protein] to kill HIV-infected cells or inhibit fusion and syncytia formation resulting subsequent to HIV-infection.

It may be important to note here that the present invention differs significantly from and has advantages over other treatment modalities of AIDS at least in the following respects.

I. It has been reported that soluble derivatives of CD4 block HIV infectivity of cells in culture, presumably by competing for the ability of the virus to bind to cell-associated CD4. Without being bound to any specific theory, it is postulated that the invention described herein acts by a different mechanism, namely by killing cells which have already been infected with HIV. In this regard, it has been reported that soluble CD4 is much less effective when added after the virus has been allowed to infect the cell, unlike the CD4-toxin which kills cells after infection has occurred. It should be noted that the hybrid toxin may also produce competitive inhibition of infectivity seen with soluble CD4 in addition to its targeted killing of HIV-infected cells.

II. Selective killing of HIV-infected cells using an immunotoxin composed of an anti-gp120 mouse monoclonal antibody chemically conjugated to protein toxin (ricin) has also been reported. However, the CD4(178)-PE40 fusion protein of the present invention possesses numerous advantages over this immunotoxin: (a) In the case of the immunotoxin the antibody used is type specific, and does not bind to gp120 from diverse isolates of HIV-1. In contrast, CD4(178)-PE40 may be used against divergent strains of HIV-1 as well as against HIV-2, since all these viruses use CD4 as the receptor. Because of this requirement for CD4 receptor specificity, it is extremely unlikely that variants of HIV, resistant to CD4-toxin hybrid proteins, will arise, whereas variants which no longer bind type-specific monoclonal antibodies often arise. (b) The immunotoxin is produced by chemical coupling procedures which are difficult to control, thereby compromising the uniformity of the conjugate and also result in low yield. In contrast, the recombinant CD4(178)-PE40 fusion protein can be produced in large quantities in a bacterial expression system using standard procedures. (c) The mouse immunoglobulin component of the immunotoxin is likely to be immunogenic in human subjects, thereby compromising its effectiveness. In contrast, with CD4-toxin fusion proteins, the targeting to gp120-expressing cells is achieved by a fragment of human CD4, which is likely to be less immunogenic in humans.

III. Selective killing of HIV-infected cells in vitro by liposomes containing diphtheria toxin fragment A has also been reported. Clearly, this is quite distinct from the fusion-protein methodology of the present invention.

Having described certain aspects of the present invention, various modifications thereof which can be

achieved by one of ordinary skill in the art, are now listed.

A. Variations in the CD4 portion. This can be achieved, for example, by differences in length of the CD4 sequence. Shorter or longer versions of the CD4 sequence can be found which can also be attached to toxins to achieve selective killing of HIV-infected cells. The length of the CD4 sequence can have important consequences for the affinity for gp120, for the relative affinities for gp120 vs. class II antigens, for the physical accessibility to different regions within the body, and for the immunogenicity. In addition, site-specific mutagenesis can be used to decrease the affinity of CD4 for normal cellular antigens, and/or increase the affinity for gp120. Such mutations would widen the window between effective therapeutic dosages and unwanted toxic side effects.

B. Variation in the toxin portion. Modifications of PE can be made. By selective mutagenesis or deletion, the immunogenicity of the PE sequence can be reduced and the potency of the hybrid toxin increased (e.g., by enhancing translocation or catalytic activity).

In addition to PE, other toxins such as ricin and diphtheria toxin fragment A could be similarly employed in context of fusion technique described herein.

C. Expression systems

Bacterial. By employing, for example, certain *E. coli* expression system, secreted forms of the hybrid toxin can be made obviating the need for denaturation/renaturation.

Eukaryotic. Mammalian, vaccinia virus, baculovirus, and yeast expression systems can also be used as advantageous expression systems as is well known to one of ordinary skill in the art.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

What is claimed is:

1. A chimeric gene which encodes a hybrid protein, wherein the hybrid protein comprises a sequence of human CD4 containing a binding site for HIV gp120 linked to a sequence of *Pseudomonas* exotoxin A essential for cell toxicity.

2. The chimeric gene of claim 1, wherein the cytotoxic protein includes sequences of *Pseudomonas* exotoxin A essential for cell toxicity.

3. The gene of claim 2 encoding CD4(178)-PE40 hybrid protein.

4. The gene of claim 3 inserted in a cloning vector.

5. The gene of claim 4 wherein said cloning vector has all the functional characteristics of ATCC deposit No. 67739.

6. The gene of claim 5 expressed in *E. coli*.

7. The chimeric gene of claim 1, wherein the sequence of human CD4 includes residues 1-178.

8. The chimeric gene of claim 7, wherein the cytotoxic protein includes sequences of *Pseudomonas* exotoxin A essential for cell toxicity.

9. The chimeric gene of claim 1, wherein the cytotoxic protein is PE40.

* * * * *

the specifically of the cytotoxic effect. HUT102 is a human T cell leukemia cell line. It is well known that murine IL4 does not bind to human cells (Ohara et al, supra; Park et al, 1987, *Proc. Natl. Acad. Sci. U.S.A.* 84:1669-1673). Therefore, this cell line was first tested because it had been shown previously to be sensitive to another chimeric toxin, IL2-PE40 (Lorberbom et al, 1988, *Proc. Natl. Acad. Sci. U.S.A.* 85:1922-1926). As shown in Table 1, IL4-PE40 had very little or no cytotoxic effect on HUT102 cells ($ID_{50} > 1000$ ng/ml). Two other human cell lines, A431 and KB, were also tested and IL4-PE40 was found not to be cytotoxic to them. On the contrary, IL4-PE40 was cytotoxic to murine cell lines, CTLL (a T cell line) and P815 (a mastocytoma cell line), which had been reported to possess IL4 receptors (Ohara et al, supra; Park et al, supra). IL4-PE40 was also cytotoxic to a murine myeloma cell line, P3X63-Ag8.653. IL4-PE40 had weak cytotoxic effects on two murine fibroblast cell lines, Swiss 3T3 and L929, but had little or not effect on NIH 3T3. The cytotoxic activity of IL4-PE40 to CTLL, P815, Swiss 3T3 and L929 was neutralized by anti-IL4 antibodies (11B11) (data not shown). IL4-PE40 asp^{553} or PE40, lacking ADP-ribosylating activity or cell binding domain respectively, had very low effects on all the cell lines listed in Table 1. These results confirm the specific cytotoxicity of IL4-PE40.

The availability of IL4-PE40 now makes it possible to suppress immune response. It has been reported that activation of B and T cells with mitogen or anti-IgM antibody produces a 5- to 10-fold increase in IL4-receptor number (Park et al, 1987, *J. Exp. Med.* 166:476-488). Hence IL4-PE40 could be utilized for immuno-suppression by depleting activated lymphocytes. IL4-PE40 could also be used for the treatment of certain tumors because it has been reported that certain tumor cell lines derived from B-lymphomas, T-Leukemias, mastocytomas and the like have relatively high number of IL4-receptors (Ohara et al, supra).

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

TABLE I

CYTOTOXIC ACTIVITY OF IL4-PE40

Cell LINE	ID_{50} , ng/ml			
	IL4-PE40	IL4-PE40 asp^{553}	PE	PE40
CT4R	17	>1000	>250	>1000
CTLL	250	>1000	80	1000
P3X63-Ag8.653	12	>1000	N.D.	>1000
P815	20	>1000	59	>1000
NIH3T3	>1000	>1000	0.86	>1000
Swiss3T3	420	>1000	0.16	>1000
L929	350	>1000	0.16	>1000
HUT102	>1000	N.D.	3.4	N.D.
A431	>1000	N.D.	1.2	N.D.
KB	>1000	N.D.	29	N.D.

The ID_{50} was calculated from protein synthesis inhibition assays measuring [3H]leucine incorporation after two days incubation with toxin. N.D.: not done.

What is claimed is:

1. A functionally active recombinant IL-4-PE40 fusion protein that selectively kills cells bearing IL-4 receptors, without killing cells lacking IL-4 receptors, wherein the fusion protein has ADP ribosylating properties.

2. The recombinant fusion protein of claim 1 produced by employing plasmid pM048 in an expression vector.

3. A composition, comprising an effective amount of the recombinant fusion protein of claim 1 and pharmaceutically acceptable carrier.

4. A mutant form of the fusion protein of claim 1 which consist of IL-4-PE40 Asp^{553} .

* * * * *